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On

"A Consideration of the Hepatic Efficiency
Tests, with special reference to the
Laevulose Tolerance Test."



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SECTION I.

INTRODUCTION.

GENERAL INTRODUCTION.

Within recent years many investigators have directed attention to the fact that much useful information should be obtainable about the existing state of many important organs of the body by accurately conducted and cautiously interpreted "function tests." Information derived from these would obviously be of inestimable value to the clinician not only for purposes of diagnosis but also for prognosis and treatment. Despite the most careful and skilful clinical examination it is not always possible to be quite certain about the nature or extent of a pathological condition. Reliable functional tests promise to be of very definite assistance in the elucidation of such problems.

Broadly speaking, a so-called "function test" to be of real value should either be based on some physiological function or else be dependent on some special property of the gland or organ under examination. The ideal test would, in the details of its technique, be independent of organs, other than the one for which it is designed. For example, if we rely on urinary analysis for testing some organ other than the kidneys

we at once introduce a potential source of fallacy in the varying functional powers of the latter. It may, of course, be possible sometimes to make sufficient correction for such factors.

Again, the test should be capable of being conducted at will in such a way that reasonably accurate measurements of efficiency may be recorded under widely varying conditions in health and disease. It is also most desirable that such tests should, if possible, be simplified, partly for the comfort of the patient and partly to render them available to the practitioner without his having to rely on the highly skilled laboratory worker for the conducting and interpreting of the test. Finally, the test should be sufficiently sensitive to divulge the smaller degrees of divergence from normal, while there is yet a possibility of arresting a pathological process before irretrievable damage, direct or indirect has resulted therefrom. It is obvious that a test which is so gross that it is merely capable of confirming a condition already evident from symptoms and physical signs is, practically speaking, superfluous, though, theoretically, it may be interesting.

It is to be expected that a series of functional tests will only attain even the more important of the above conditions by a gradual process of evolution.

The majority of "function tests" so far devised are concerned with abdominal organs, those dealing with the stomach, pancreas, liver and kidneys being the best recognised. The number suggested and the ingenuity exhibited in devising them are both very considerable. Unfortunately, many of them seem to be of little value, being based on purely hypothetical physiological grounds; or because they are too gross, etc., etc. Others, whilst not as yet definitely and fully substantiated, promise to develop into useful tests. A very few have already won wide recognition. As an example of this select group we may cite the Fractional Test Meal as a means of assessing the functioning capabilities of the stomach. Though sometimes yielding an anomalous result, it probably agrees more nearly with the conditions above enumerated than does any other function test as yet devised.

Quite recently many efforts have been made to provide tests for assessing the functional capacity of the liver in health and in disease. It is with this group that the writer is especially concerned in the present thesis.

In regard to the liver, the number of tests so far suggested is very great. It is, however,

obvious that a goodly proportion of them must be included in the first of the above three categories. Therefore, only a restricted number of the more rationally devised and better supported ones will be selected for discussion, with the proviso that certain of these, even, may ultimately be abandoned also.

Attempts to estimate the functioning efficiency of the liver are faced with many grave difficulties.

Firstly, the number and nature of its physiological functions are but indefinitely understood. It is certain that there are several and it seems possible that even more may yet be discovered; for example, some writers (1) claim that there is a hepatic internal secretion which profoundly influences the blood pressure. If this statement is justified by future work, a very great advance in our medical knowledge will have been achieved.

Secondly, impairment of one liver function must not, of necessity, be taken as implying a corresponding degree of inefficiency of any or all of the remaining ones, unless weighty evidence can be produced to support such an assumption. On theoretical grounds, at least, it seems improbable. Therefore, a multiplicity of tests for the full investigation of hepatic efficiency appears to be inevitable.

Thirdly, the accepted fact that the liver possesses an almost incredible reserve capacity introduces an obviously serious problem, suggesting, as it does, that even a fairly considerable amount of damage may escape full detection by means of these tests. Actual experiments have demonstrated that as much as 70 per cent, and perhaps even more, of the liver may be excised without leading to any decided effect on the general condition of an animal, beyond the shock of the operation (2).

Fourthly, a further complication lies in the quite remarkable power of compensatory hyperplasia, inherent in hepatic tissue. This would be a factor to keep in mind, particularly in carrying out a series of "progress tests", and in attempting comparisons between different cases.

Fifthly, the liver is a difficult organ to investigate, because it is scarcely possible to collect its specific secretion, the bile, quantitatively.

Sixthly, many of the available tests are dependent also on other organs. In some, it is necessary to administer substances by mouth in order to ensure their passing into the liver (by way of the portal bloodstream), there to be acted upon in accordance with the

physiological principles upon which the particular test is founded. This necessarily introduces the factor of intestinal absorption. Apart from possible normal variations it is certain that many of the cases likely to come under examination, e.g. cirrhosis, catarrhal jaundice, chronic passive congestion, etc., are just the ones in which we might expect faulty absorption owing to the congested and sluggish nature of the portal circulation. Yet other tests are based on urinary findings. This involves a knowledge of the integrity or otherwise of the renal function, so that it may be necessary to perform parallel renal efficiency tests. To some extent, therefore, the advance of the hepatic tests depends on progress in investigating kidney efficiency.

Seventhly, since any given substance remains in the liver cell for a relatively short period, and, especially in disease, fluctuates rather widely and rhythmically, its presence in a low concentration may still be consistent with functional adequacy (3).

Finally, the tests at present under review in many instances demand considerable time and skill as well as rather costly apparatus. These features largely restrict their application to the hospital patient.

With due regard to these several difficulties, a group of tests, as far as possible covering the various hepatic functions so far investigated, has been selected for discussion in the present thesis.

Special attention is devoted to the Laevulose Tolerance Test, because early in this investigation it appealed to the writer as being based on sounder physiological principles than many of the others mentioned. Moreover, we believe that it shows more promise of developing into a test of wider application in diagnosis, prognosis and treatment, than do most of the other hepatic functional tests.

It was the classical work of Claude Bernard in 1857 that may be said to have first suggested the feasibility of assessing the functional capacity of the liver, inasmuch as it inspired a considerable amount of research into the principles governing carbohydrate metabolism in the animal body. It was the natural outcome of this that the possibility of establishing Carbohydrate Tests for the purpose was the first to be exploited. It is true that no progress of any note was made for nearly half a century, but in the earlier portion of this period, investigations were undertaken largely by pathologists and physiologists who, naturally enough, were more immediately concerned with carbohydrate metabolism in general rather than with the application of their findings to the liver test problem in particular. In the last quarter of a century, by which time clinicians were beginning to participate also, attention became focussed on the subject by a protracted and, at times, spirited controversy in which there developed a definite line of cleavage between the French and German schools. The question at issue was the relation of the liver to glycosuria subsequent to the ingestion of carbohydrates. The German group, relying mostly on large doses of glucose, were not able to prove that a reduction of sugar tolerance

occurred in their cases of liver disease. The French investigators, with whom cane-sugar, sometimes in relatively huge doses, was the favoured medium, countered this by recording glycosuria in numbers of hepatic cases. They were correspondingly enthusiastic in regard to the clinical possibilities of their method. This deadlock lasted until the beginning of the present century when Strauss began to record his impressions in a series of important papers dating from 1898. He very pertinently suggested that some of the confusion resulted from the adoption of different sugars and of varying dosage by the different workers. He also made the important observation that glucose was quite unsuitable for the purpose as it could be dealt with elsewhere in the body as well as in the liver. He supported this contention with careful experiments on frogs, in which he noted that the glucose tolerance following hepatectomy was not very much less than that preceding the operation. Applying this principle to the human subject he was only able to find a transient glycosuria in two out of 38 cases of liver disease. He also investigated the French test and attributed the higher percentage of positive results with it to the laevulose portion of the cane-sugar. His work culminated in 1901 (4) in the proposal that laevulose was the sugar most suitable for investigating the liver function.

Sachs (5) had already paved the way towards its adoption, for, three years previously, he had definitely demonstrated, also in frogs, that the tolerance for laevulose was much reduced after extirpation of the liver. Strauss applied his laevulose test to several hepatic cases and was able to demonstrate a high percentage of positive urinary findings.

His test soon became well known in Germany and for some years a good deal was written about it, but fuller experience gradually convinced his successors that this method, too, was wanting in consistency.

In 1906 Bauer (6) attempted to introduce galactose for the purpose of testing the liver in Catarrhal Jaundice specially, contending that galactosuria would result in this condition, and in a few cases of cirrhosis but not otherwise. His method did not gain much of a following. In 1912 Churchman (7) concluded from his exact work that Strauss's Test was unreliable. He had even graded his laevulose dosage in accordance with the body weight of the patient being investigated.

With the exception of one valuable paper published by Shirokauer early in 1913, this resume covers the history of the carbohydrate liver tests up to the end of 1913. This paper we think is so important that

it begins a new chapter in the investigation of the liver.

We pause, therefore, to consider certain other hepatic tests proposed from time to time by various writers.

Urobilinuria had been considered by von Jaksch (8) as long ago as 1892 to indicate hepatic disorder. The test for it was essentially qualitative.

In the interval up to 1913 a few investigators had recorded their findings with the test, and they mostly confirmed the original observation. Bauer (9) in 1905 found it in the majority of hepatic diseases. According to Fischer (10) it was present in the pre-icteric stage of acute catarrhal jaundice. In 1911 Falk and Saxl (11) announced their studied opinion that it was a very sensitive hepatic test, being positive in instances where the damage was too slight to influence the carbohydrate or nitrogen partition methods in a positive direction.

The Fibrinogen Test resulted from the observation of Doyon and Kareff (12) in 1904 and of Nolf (13) in 1905 that this substance could not be detected in the blood very soon after excision of the liver. Doyon demonstrated its reduction in animals poisoned with chloroform. Whipple (14) reviving the test in 1912, found its amount decreased in some forms of gross liver damage but his results were not consistent.

The Urinary Amino-Acid Content was found to be increased in many types of hepatic disease by Glaessner

(15) in 1907. He also pointed out that the amino-acid N/ total N co-efficient rose in value in these circumstances.

In 1911 Falk and Saxl attempted to estimate the ureogenetic efficiency of the liver by administering glycocoll to their patients on the assumption that the disease-damaged organ would fail to turn this amino-acid into urea and that the unconverted fraction could be identified by urinary examination.

The Dye Tests for investigating the excretory powers of the organ date from the observations of Rowntree and Abel (16) in 1909 that the drug phenoltetrachlorophthalein, prepared by Arndoff and Black of Cornell in the preceding year, was mostly excreted in the bile, just as its relative, phenolsulphonephthalein, was largely eliminated in the urine. In 1913 Rowntree and his colleagues began to publish the results of their preliminary trial of the new method. They endeavoured to reclaim it from the faeces quantitatively and believed that the test would be a useful one.

The above outline of the hepatic efficiency tests carries us up to 1913 and it must be conceded that very little material progress had as yet been made in the direction of furnishing the clinician with tests that he could rely on to help him in deciding perplexing problems in diagnosis, etc.

The carbohydrate tests had excited a large amount of active research, clinical and otherwise, but much of this had rather tended to discredit them practically. The urobilinogen test, looked upon by some as an almost specific indication of liver disorder, had been too little worked at to encourage confidence.

The Nitrogen partition methods had appeared but, so far, had appealed to only a very few workers. A few new tests were in course of trial by a small group of American investigators. These included the dye test, which was very hopefully regarded by its sponsors.

From 1913 up to the present time an extensive amount of new work on the subject has made its appearance from time to time, especially in America and on the Continent. Many new tests have been proposed and hardly any known field of hepatic activity has been left unexplored in the effort to find a test or tests that will render easier the task of the clinician faced with the difficult problems in which hepatic pathology abounds. Indeed this branch of bio-chemistry has now expanded into a vast and complex "chemical semiology", as Chauffard has aptly termed it.

Shirokauer (17) in the paper above alluded to made a very important advance when he introduced the modification of estimating the blood-sugar content instead of relying on urinary findings in the Laevulose Test.

He showed that the blood-sugar concentration, unaffected by laevulose in health, showed a definite increase in his cases of liver disease. The urinary output of the sugar was not necessarily parallel. Since his time the blood-sugar method has entirely superseded the older method.

A number of papers have appeared on the modified test and these will be referred to when considering the Laevulose method in detail.

The dye tests have also been much worked at. Various refinements of technique have been developed, so that most writers now use blood estimations in place of the various cumbersome methods formerly in vogue. Several new dyes have more recently been proposed, and are in course of investigation.

The Nitrogen-Partition methods have not advanced much in favour. During the last decade various new tests have been suggested, including the important method which Van den Bergh introduced in 1918 for detecting bilirubin in the blood serum.

The modern views on jaundice are in a large degree due to the work of this investigator. He "more than anyone else has transferred our outlook on jaundice away from the colour of the skin and presence or absence of bile pigment in the urine and faeces, to the changes occurring in the blood-serum. He has done this by his

success with a method of detecting bilirubin in blood-serum which is more accurate and delicate than any test previously employed for this purpose in clinical or experimental work." (18).

A simple method for estimating the amount of urobilin in the urine was introduced by Marcussen and Hansen (19), also in 1918.

Widal and his associates (20) in 1920 published a test, under the title of "the digestive haemoclasia" or "the haemoclastic crisis", which they considered of considerable value for estimating the function of the liver. This test has had a large following in France.

Tests based on the glycuronic ~~acid~~ content of the urine following the administration of certain drugs have also been introduced as a means of assessing the antitoxic or conjugating capacity of the organ. The more important of these various methods will be considered in detail in the sequel.

SECTION III

CLASSIFICATION OF THE TESTS SELECTED

FOR DISCUSSION.

Reference has already been made to an important principle that should govern the investigation of hepatic efficiency, namely, that it must not be taken for granted that the impairment of one of the many functions of the liver necessarily infers that all, some or even any of the remainder are correspondingly involved.

That some form of classification of the various tests should, therefore, be adopted at the outset is advisable, partly to facilitate reference, comparison and the maintenance of continuity in the discussion and partly to keep the above principle in the foreground.

As the majority of writers on the subject have confined themselves to one or at most two or three of the tests, the question of classification hardly arose. But in recent works, wherein a review of the whole subject has been attempted, the method has usually been to classify on a physiological basis. This appeals to the writer as being the most scientific method and he has, therefore, adopted it, after making some minor alterations in the general scheme. Owing, however, to the comparative obscurity still investing much of the hepatic physiology, any such scheme must remain subject to readjustment. Gruner's (3) classification is very usefully drawn up in the form of a diagram (Fig. 1) "where the various

functions of the liver are represented as 'powers' within one single cell, the 'liver unit' (the principle of histological, physiological units), each of these powers comprising several chemical processes." He then proceeds to tabulate the 'powers' or functions with their corresponding tests as follows:-

Name of Test	Power Tested.
Haemoclastic Crisis	Proteopexic
Nitrogen-Partition Test	Desaminating, Uricolytic
Phenoltetrachlorphthalien Methylene Blue Camphor Test	Toxicopexic, Uricolytic
Van Den Bergh's Test Urobilin Test	Chromopexic
Blood Cholesterol	Cholaligenic
Laevulose Test Diastase Test	Glycopexic
Fat in stools Lipase Test	Adipopexic

Anderson and Spriggs (21) suggest the following alternative classification:-

A. CONSTRUCTIVE FUNCTIONS.

1. Secretion of bile.
2. Formation of glycogen in carbohydrate metabolism.
3. " " urea in nitrogen metabolism.
4. " " fibrinogen.
5. " " antithrombin

B. DESTRUCTIVE FUNCTIONS.

6. Destruction of red blood corpuscles.
7. Detoxication.
 - (a) Formation of conjugate sulphates and glucuronates.
 - (b) Withdrawal of toxins.

Neither of these appeals to the writer as being entirely satisfactory for the purposes in view. Both are incomplete. The first is rather lacking in the element of continuity. The second includes functions, whose investigation by efficiency tests is not feasible in the present state of our knowledge.

The classification finally adopted is outlined below. Excepting certain modifications, it is substantially the one drawn up by Beaumont and Dodds (22).

CLASSIFICATION OF LIVER EFFICIENCY TESTS ADOPTED
IN THIS THESIS.

I. Tests based on Metabolic Functions.

(a) Carbohydrate.

Laevulose Test (Strauss and Shirokauer)

Galactose Test (Bauer)

(b) Protein.

Nitrogen-Partition Tests (Glaessner, Etc.)

"Haemoclastic Crisis" (Widal)

(c) Fat.

Fat Droplets in Blood (Brulé)

Lipase Test (Whipple)

II. Tests based on Pigmentary Functions.

Tests for bile pigments and precursors in urine,
faeces and blood.

Van den Burgh's Test.

Urobilin Test (von Jaksch)

III. Tests based on Secretory Functions.

Tests for bile salts in urine

Blood-Cholesterol Content.

IV. Tests based on Antitoxic Functions.

Glycuronic Acid in Urine (Roger) after various
drugs (Roch, Destree and van Doren, etc. etc.).

V. Tests based on Excretory Functions.

The Various Dye Tests.

VI. Tests based on Haemopoietic Functions.

Fibrinogen content of Blood (Doyon, Whipple etc).

Coagulation time of Blood.

SECTION IV.

DETAILED CONSIDERATION OF

SELECTED HEPATIC FUNCTION TESTS.

I. TESTS BASED ON INVESTIGATION
OF METABOLIC FUNCTIONS.

- (A) Carbohydrate.
- (B) Protein.
- (C) Fat.

(A) TESTS BASED ON INVESTIGATION OF
CARBOHYDRATE METABOLISM.

That the liver fulfils an important role in carbohydrate metabolism has long been recognised and, as we have already noted, it was this department of hepatic activity that was the first to be utilised in the endeavour to establish tests for estimating the functional efficiency of the organ.

THEORETICAL BASIS OF TESTS.

The carbohydrates of the food are believed to be ultimately absorbed from the intestine in the form of the simple monosaccharides - especially glucose. These are conveyed to the liver in the portal blood-stream, there to be polymerised and stored as glycogen. Re-conversion into dextrose subsequently takes place in accordance with the demands of the body-tissues. It seems that under ordinary conditions just sufficient is broken down to maintain the blood-sugar concentration at a fairly constant level (in round figures, about 100 mgrm. per 100 c.c.). The various tissues abstract from the blood such amounts of glucose as they require,

the deficit being at once made good by the liver from its glycogen store. The glucose is oxidised into carbon-dioxide and water, so furnishing the requisite energy for the various tissue activities of the organism. This storing capacity of the liver serves at least a two-fold purpose.

In the first place we may regard the liver as a "buffer" between the intestine, which absorbs as much sugar as it can, on the one side, and the systemic blood-stream, wherein a reasonably even sugar-concentration is to be maintained on the other. It is easy to imagine that, in the absence of such an intermediary, the blood-sugar content would fluctuate widely in response to the amount and frequency of carbohydrate absorption.

Secondly, there is set up a reserve ready to be drawn upon when the tissue-demand for glucose temporarily outbids its supply. By these means it is, therefore, possible, under ordinary conditions, for an equilibrium in the blood-sugar concentration to be maintained. It is also possible that some change in the glucose is brought about to enable assimilation to take place. This point will be further referred to later.

With the advent of modern methods of accurate blood analysis it has become evident that the normally

functioning liver does not accept and store all monosaccharides with the same facility. According to Maclean (23) "all the ordinary sugars, with one exception, give definite blood-sugar curves after ingestion". In the case of glucose "the sensitiveness of the blood-sugar content to the ingestion of even very small amounts is remarkable", for a distinct, if slight, rise follows a dose of even 5 grms. The height attained by the curve gradually increases with the dose until its maximum peak is reached after about 20-25 grms. Thereafter the increase of dose fails to produce a further elevation of the blood-sugar content, though it tends to lengthen its duration. In the normal subject, to which the above applies, this maximum may be taken as about 0.160-0.170 per cent, and is reached usually within one hour - most often in 30-40 minutes. Once this maximum is reached, the concentration rapidly declines, until after a further interval of about the same duration, the level reached is below that at which the experiment began.

In Fig. 2 these various features of the blood-sugar content after the ingestion of glucose in varying doses are represented diagrammatically. These facts refer to young healthy adults. Spence (24) has shown that in children under three years of age the

maximum reached rarely exceeds 0.120-0.130 per cent. After three years the curve approximates to the adult type. At the other extreme of life we find that, as age advances, the curve tends to be lengthened, so that a concentration of about 0.140 per cent after two hours would not necessarily be regarded as out of the ordinary in a man of 65 years. It is merely an index of his declining metabolic activity.

Lactose yields a more prolonged rise with a rather lower maximum than glucose (Fig. 3).

Maltose closely resembles glucose in the details of its curve. (23).

Laevulose (fructose), however, furnishes a remarkable contrast to these other sugars. Maclean and de Wesselow (25) claim that it is "the only sugar" in ordinary use which causes no appreciable rise in the blood-sugar concentration when administered in the usual doses employed in these investigations. In other words, the curve obtained after ingestion of laevulose by the normal person is represented by a practically straight line at the level of the blood-sugar content before administration or at most by a curve showing minor oscillations which might equally as easily be obtained without giving laevulose at all. This distinctive property of

laevulose has been confirmed by all writers on this subject. Results obtained from our own series of normal cases (quoted on p. 40) are in entire agreement with the above observations. Included in the list are figures for two healthy persons beyond the age of 65 years. In neither do we detect any special feature.

Of galactose Maclean writes that "the curve obtained resembles that of glucose" (23) and again that "galactose easily produces glycosuria" (23). The reports on this sugar are, however, inconsistent. Kahler and Machold had already demonstrated that the rise in blood-sugar content following its ingestion was comparatively inappreciable. This has been supported by later authors (26).

It is of considerable importance that the sample used should be pure (27) and it is possible that impurities were responsible for the conflicting reports above enumerated.

The sugars above referred to can, therefore, be divided into two groups (1) those causing a sharp rise in the sugar-content of the blood, (2) Laevulose, which causes no appreciable increase when administered to the normal subject. It would appear that in the case of this sugar glycogenesis is particularly active and

that the liver is easily able to deal with the dosage quantities without allowing any easily detectable amount to pass through into the general circulation. Sir H. Rolleston (28) has pointed out that laevulose is the only carbohydrate whose metabolism is carried out entirely in the liver. A series of important experiments conducted by Woodyat, Sansum and Wilder (29) lends considerable support to this view. By carefully controlled intravenous administration of laevulose they demonstrated that the tolerance of the normal individual for laevulose was very low. A considerable proportion of the sugar so introduced was at once excreted by the kidneys, yet a considerable amount can be easily tolerated when given by mouth. In the former experiment, the laevulose is introduced directly into the systemic circulation. In the latter, the laevulose absorbed from the alimentary canal of necessity passes to the liver. The logical interpretation of these experiments is that only in the liver can laevulose be polymerised and stored. In the same way glucose was investigated. They found that the normal subject could use up 0.8 to 0.9 grm. per kilo of body weight per hour without any glycosuria developing. Evidently the metabolism of glucose is not solely confined to the liver, as was originally pointed out by Strauss. Galactose gave

results similar to those of laevulose, whilst in the case of lactose the tolerance was almost nil.

Since, therefore, the blood-sugar content can be readily shown to be unaffected by the ingestion of a given amount of laevulose, owing to the ready glycogenetic response of the healthy liver, it follows that we would expect a delayed or defective reaction in the case of a liver which has been damaged by pathological processes. We would further anticipate that the laevulose thus inadequately dealt with would pass on into the systemic blood in greater or lesser amounts and that this would be revealed by an increase in the blood-sugar concentration. Upon these premises the laevulose and galactose tests are based.

Before leaving this part of the subject it is necessary to refer also to the remarkable theory propounded by Cammidge, Forsyth and Howard (30) to account for the blood-sugar rise after food. They claim that this is caused by changes in the reaction of the blood due to the absorption of salts formed from the digestive juices and resulting in an increased breaking down of pre-formed glycogen stored in the liver. This increased glycogenolysis, they believe, is ultimately caused by the stimulation of the diastatic ferment of the liver,

this in turn being induced by three factors, viz:

(a) alteration of the H-ion concentration of the blood,
 (b) increased permeability of the liver cells to Na Cl,
 (c) removal or lowering of the inhibitory influence of the pancreas. Their views are epitomised in Fig. 4, which is reproduced from their paper. They find in their experimental work justification for the following unusual statements:-

(1) A purely protein meal has little, if any, less power than has a carbohydrate one of raising the blood-sugar content.

(2) In a healthy subject a mixed meal causes a fall in blood-sugar during the first half-hour, coinciding with "the alkaline tide" of the urine and passing of sodium-ions from the stomach into the blood.

(3) That the normal sugar curve following such a meal consists of this fall for thirty minutes or so, followed by a rise to a maximum at three hours, with, finally, a slow fall until the original fasting level is re-established at about the fifth or sixth hour.

Somewhat similar views had been expressed by Langfeldt in 1921 (31) but otherwise, so far as we have been able to ascertain, the bulk of opinion is in agreement with the following principles:-

(1) That a protein meal does not lead to a significant rise in the blood-sugar curve.

(2) That a mixed meal, as would be expected from its glucose fraction, causes an immediate rise, and that the curve tends to follow the characteristics of the glucose one as already enunciated.

Spence and Brett (32) further point out that this theory ignores the fact "that the liver is not richer in diastase than are the muscles, lungs, kidneys and other organs, that it is extremely improbable that absorption of acid is so great as to overcome the regulating mechanism in the blood and so produce an actual increase in the H-ion concentration, and that given the presence of a certain minimum of salts for diastatic action, a mere excess of sodium chloride in the liver cells will not increase that action in proportion to the excess."

These writers have also carried out an experiment in which, after making blood-sugar estimations subsequent to the ingestion of glucose (50 grm) in the usual way, they inserted an Einhorn tube so that it reached into the jejunum (confirming its presence by X-rays) and then repeated the estimation after introducing the glucose

directly into the small bowel. Their figures which show a close parallelism are quoted from their paper:-

Time after	B. S. per cent	
	Into Stomach	Into Jejunum
20 Mins.	.154%	.136%
30	.153	.1167
40	.161	.161
60	.132	.117
100	.102	.102

This method would appear to eliminate the influence of the digestive juices in the case of the glucose introduced directly into the jejunum of the fasting subject. Furthermore, they found that sugar given by mouth in strongly acid and in strongly alkaline solutions produced the same curve as that given in the ordinary way in water.

It is, therefore, concluded that there is little to support the newer theory advanced by Cammidge and his collaborators, and that for the purposes of this test the physiological principles enunciated above have the bulk of evidence in their favour.

SUMMARY OF MORE IMPORTANT LITERATURE.

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The Laevulose tolerance test for estimating the glycogenic "power" of the liver was introduced by Strauss (4) some twenty-six years ago. As already stated the special indication for using laevulose arose out of the experimental work conducted by Sachs (5) on hepatectomised frogs, in which he discovered a striking intolerance for this particular sugar, a result which he was unable to reproduce with glucose, arabinose or galactose. Strauss administered 100 grm. of laevulose by mouth to the fasting patient, and then collected the urine excreted for at least four hours afterwards. This he tested by Trommer's and Seliwanoff's methods as well as by fermentation and with the polarimeter. By these means, he was able to record laevulosuria in 90 per cent of his hepatic series (including "cirrhosis", seventeen cases; "carcinoma", three cases; "complete obstruction", four cases; "amyloid disease", one case; "diabetes with cirrhosis", one case), whereas in fifty-eight normal controls (which included also diseases clinically not involving the liver) he obtained but six positive results, which, indeed, he attributed to latent hepatism inasmuch as they occurred in cases of gout, pneumonia, obesity, etc.

Ferranini (33) followed this up by contrasting his results with both fructose and glucose in sixteen cases of liver complaints. He confirmed the value of the laevulose method. Bruining (34) obtained ninety per cent of positive findings in his hepatic series. A strong protest against the method was entered by Landsberg (35) who not only found laevulosuria in less than half of his series of patients with liver disorders but also proved its presence in more than half of a small normal series. He considered that tolerance for this sugar was a variable factor, even in the healthy subject.

Chajes (36), Sabatowski (37), von Halasz (38) all obtained results more or less in harmony with Strauss's views.

Falk and Saxl (11), on collecting a large number of case records, found that the positive findings in hepatic disease numbered about 80 per cent. Churchman (7) recorded nine positive results amongst thirty-five normal subjects, and also found a few negative ones in undoubted liver disease. He believes that neither positive nor negative findings could be relied on. Wörner and Reiss (39) concluded from their experiments on "alimentary galactosuria and laevulosuria" that the latter could only be accepted as indicative of hepatic

disorder when more than 0.100 grm. was identified in the urine. Even with this reservation they exhort the observer to exercise considerable caution in associating the laevulosuria with liver disease. Shirokauer (17) was the first to utilise blood-sugar estimation in the test. He made the important assertion that the blood-sugar concentration showed no appreciable augmentation consequent upon the ingestion of laevulose by the normal fasting individual. In cases of established hepatic disease, however, he found a distinct rise. He retained the 100 grm. dosage and made a single estimation one hour later. Applying Bertrand's method to the serum, he noted values as high as 0.190 per cent in liver disease. Laevulosuria, he noted, was simultaneously present in the majority of cases but did not necessarily show a parallel with the blood-sugar findings. Whilst a great step forward was made by Shirokauer, the fact that only one blood estimation was made must count as a deficiency in his method. The introduction of Bang's micro-chemical method of estimating the blood-sugar content afforded a means of observing its changes over any desired period. Bergmark (40) made use of it to confirm Shirokauer's contention that laevulose differed from glucose in not causing an appreciable rise after ingestion by normal

persons. As his observations extended over periods of two to three hours, they are much more valuable than those of his predecessor. In 1920 MacLean and De Wesselow (25) verified this finding. In the same year Isaac (41), in investigations designed to estimate the position of laevulose in carbohydrate metabolism, also demonstrated that ingestion of this sugar leads to no appreciable rise in blood-sugar in health. He claimed that he could estimate the relative amounts of laevulose and glucose in the blood serum following 100 grms. of the former taken by mouth. Glucose formed very much the higher proportion and laevulose quickly disappeared. In liver derangement, on the contrary, he found not only that the total blood-sugar content rose to a considerably higher level, but also that the laevulose fraction was much greater and persisted in the blood for a longer period.

The possibility of distinguishing between the two sugars has been denied by Winter and Smith (42) who were unable to prove laevulose as such to be present in the blood. They are of the opinion that the form in which sugar circulates in the blood stream is γ -glucose, derived from "a common enolic form" and, further, that the production of this form is an essential preliminary

to assimilation. They account for the more rapid conversion and storage of laevulose, on the grounds that it is probably more readily turned into this enolic form.

A pathological condition of the liver would interfere with the production of the substance (? enzyme), responsible for the change, with the result that some of the laevulose would escape conversion into glycogen and thus reach the systemic blood-stream, there to cause the elevation of the sugar curve seen in many hepatic diseases.

Bornstein and Holm (43) found a definite rise in the respiratory quotient immediately after laevulose ingestion. This was deferred for forty-five minutes when glucose was substituted. They conclude from their experiments that glucose undergoes conversion into some form of sugar akin to laevulose before it is metabolised.

Spence and Brett (32) in recording their experience with the test, especially in cases of toxic salvarsan hepatitis, conclude that it is a valuable indication of liver inefficiency and that, "generally speaking the evidence suggests that the height and length of the blood-sugar curve are in proportion to the degree of liver damage present." They object to the 100 grms. dose of laevulose, so frequently employed hitherto, on the grounds that even normal individuals may respond to it with a definite rise. This dose "seems to be beyond

the capability of even the healthy liver," and quote a normal case which yielded the following figures:-

	100 grm.	50 grm.
Fasting	.089	No appreciable rise
$\frac{1}{2}$ hr. after	.117	
1 " "	.153	
$1\frac{1}{2}$ " "	.118	

They, therefore, utilised the smaller dosage in their investigations. They also agree that urinary estimations are valueless. Langdon Brown (44) thinks that the test "is one of the best we have for hepatic efficiency" whilst Langmead (44) sums it up as mainly of use in "acute destruction of the liver cells", and as not being dependable in chronic cases.

Mackenzie Wallis (44) has found it a "good and delicate" method of estimating damage inflicted by the Salvarsan group of drugs. Tallerman (45) finds that the test "is a most useful one, but that the normal variations appear to be rather greater than previously stated." He sees no advantage in regulating the dose of laevulose according to the body weight and considers 45 grms. a satisfactory dose. He calculates that the

renal threshold for laevulose is normally in the neighbourhood of 0.02 per cent (in terms of blood concentration) and agrees that urinary estimations are valueless. Tallerman applied the test to a number of normally pregnant women and found no evidence to suggest that the liver was involved. The renal threshold for laevulose became, however, "practically non-existent."

Anderson (21) writes that the "test is valuable but fails to detect small degrees of liver inefficiency. At Ruthin Castle they had obtained "negative results more often than not where liver derangement was thought to be present on clinical evidence."

Within the current year King (46) has published his findings in a comprehensive series of diseases of the liver and biliary tract as well as in ten normal controls. He has secured "consistent results" throughout. He finds that there is an atypical curve in hepatic disease and concludes that the test is of "considerable value as an index of the functional capacity of the liver."

TECHNIQUE OF TEST.

TECHNIQUE OF TEST.

The patient was allowed a very light breakfast first thing in the morning.

About four or five hours later a sample of blood (0.2 cc.) was received into a specially graduated pipette (as used by MacLean (23)) from a pin-prick in the finger.

The dose of 50 grms. of laevulose in 100 c.c. of water was then given.

Blood samples of the same volume were then collected at each half-hour interval up to two hours afterwards.

The estimations were carried out by MacLean's method (23).

DOSAGE: The smaller dosage was adopted for the following reasons:-

(1) It has proved satisfactory in the hands of various workers (e.g. 32, 45, 46).

(2) There is a reduced possibility of some leaking through in the event of the liver being already well-stocked with glycogen - this probably being the explanation of the observation by Spence and Brett (32) that

100 grms. caused a distinct rise in the curve (which was not observed with the smaller dose) in one of their cases quoted above.

(3) There is less chance of nausea, etc., supervening and the volume of solution taken is less.

(4) The smallest possible dose is indicated in view of MacLeod's objection that these tests are "of a highly unphysiological nature, for the rapid, sudden and marked increase in the concentration of sugar in the blood (~~hyper-~~ glycaemia), which does not occur when the sugar is slowly absorbed, as is the case following the ingestion of a normal meal" (48). It is true that these remarks would refer to glucose more particularly than to laevulose, but MacLeod makes no differentiation. The possibility of such a rise with laevulose, however, even if not very great, would seem to be more likely to occur with a damaged liver. There would thus be introduced an exaggeration of the curve rise.

(5) The smaller dose is more economical.

RESULTS WITH LAEVULOSE TEST.FIRST SERIES.

(Apparently healthy and normal adults).

No.	Sex.	Age.	Blood Sugar Percentage					Max Rise in mgrm
			Fasting level	$\frac{1}{2}$ hour after L.	1 hour after L.	$1\frac{1}{2}$ hours after L.	2 hours after L.	
1	F	18	0.096	0.101	0.098	0.095	0.093	5
2	M	24	0.089	0.092	0.100	0.090	0.089	11
3	M	38	0.101	0.104	0.103	0.101	0.100	3
4	M	49	0.090	0.098	0.096	0.091	0.086	8
5	M	65+	0.109	0.116	0.114	0.111	0.110	7
6	F	71	0.102	0.112	0.106	0.104	0.101	10
Averages			0.098	0.104	0.103	0.099	0.097	6 ⁷

A curve based on these averages is shown in Fig. 6.

SECOND SERIES.

(Patients with diseases not clinically
involving the liver).

No.	Disease	Blood Sugar Percentage.				
		Fasting	$\frac{1}{2}$ hour after L.	1 hour after L.	$1\frac{1}{2}$ hours after L.	2 hours after L.
1	Gastric Ulcer	0.110	0.116	0.118	0.112	0.108
2	Gastric Ulcer	0.104	0.109	0.105	0.107	0.104
3	Duodenal Ulcer	0.093	0.102	0.100	0.091	0.095
4	Chronic Gastritis	0.084	0.096	0.091	0.089	0.086
5	Appendicitis	0.112	0.117	0.120	0.114	0.110
6	Appendicitis	0.099	0.106	0.104	0.100	0.099
7	Mediastinal Tumour	0.108	0.112	0.110	0.108	0.107
8	Mitral Incompetence	0.107	0.114	0.112	0.101	0.104
9	Pericarditis	0.081	0.089	0.092	0.090	0.086
10	Chronic Nephritis	0.113	0.118	0.117	0.114	0.109

THIRD SERIES.

(Patients with diseases involving Liver
or Biliary Tract).

No.	Disease	Blood Sugar Percentage				
		Fasting level	$\frac{1}{2}$ hour after L.	1 hour after L.	$1\frac{1}{2}$ hours after L.	2 hours after L.
11	Acute Catarrhal Jaundice Adm.	0.101	0.124	0.137	0.127	0.114
	Disch.	0.096	0.103	0.102	0.099	0.097
12	Acute Catarrhal Jaundice Adm.	0.087	0.128	0.142	0.132	0.119
	Disch.	0.094	0.104	0.101	0.096	0.092
13	Portal Cirrhosis	0.091	0.125	0.148	0.164	0.152
14	Portal Cirrhosis	0.103	0.142	0.176	0.190	0.169
15	Gumma of Liver	0.107	0.113	0.110	0.108	0.105
16	Toxic Salvarsan Hepatitis	0.118	0.141	0.158	0.161	0.155
17	Carcinoma of Stomach with hepatic metastases	0.082	0.097	0.113	0.102	0.100
18	Cholelithiasis	0.109	0.121	0.119	0.110	0.108
19	Cholelithiasis	0.115	0.123	0.134	0.126	0.120
20	Obstructive Jaundice. (Carcinoma - Head of Pancreas)	0.094	0.117	0.139	0.125	0.109
21	Carcinoma of Gall Bladder with hepatic invasion	0.112	0.126	0.131	0.119	0.115
22	Obstructive Gall-Stone Jaundice	0.104	0.113	0.107	0.102	0.104
These results are reproduced graphically in Fig. 6.						
23	A case of Pernicious Anaemia	gave the following results (Fig. 7)				
		0.101	0.134	0.161	0.148	0.125

DISCUSSION OF TEST.

DISCUSSION OF TEST.

The first question to be considered in connection with the sugar tolerance tests as a means of gauging the glycogenic functional efficiency of the liver is that of urinary versus blood estimations. For several years after the introduction of the test the urinary method was relied on entirely. The published results, including those relating to the normal controls, as well as those obtained in cases of avowed hepatic derangement, were often quite conflicting. Shirokauer in 1913 was the pioneer in making a dual set of observations. In contrasting his blood findings with the corresponding urinary ones, he found a lack of parallelism, which told heavily against the urinary method. Since the publication of these observations practically all workers have utilized the blood technique, though many have carried out parallel urinary estimations by various methods (e.g. Seliwanoff's Test for qualitative purposes and Bertrand's and the Safranin Tests for quantitative work).

In the normal controls, the unanimity of findings by the blood methods has been remarkable, whereas the discordance with the urinary ones has been widely confirmed.

Utilising urinary analysis for studying the results of functional phenomena consequent upon the ingestion of carbohydrates necessarily involves three factors, viz:-

(1) rate of absorption from the bowel into portal blood-stream.

(2) the glycogenic and glycogenetic powers of the subject, and

(3) the excretory capabilities of the kidneys.

As the object of such study is to obtain accurate information relating to the second of these, it would appear that potential sources of fallacy reside in the other two.

It has, however, been demonstrated that the rate of absorption of pure glucose by the normal person is remarkably constant (4%), and presumably this is true of laevulose also. Indeed, on comparing the normal blood-sugar curves published by the various authors after the oral administration of laevulose we are at once struck by their uniformity. Our own small series of normals (p. 40) agrees with these findings. Furthermore, in our second list (p. 41) in which we deliberately included gastro-intestinal conditions, we have again been unable to detect any significant aberration from normal. It is probably true that a certain proportion of patients

are unable to drink the laevulose solution without its leading to such unpleasant sequelae as nausea or vomiting and diarrhoea. None of our cases, however, exhibited any discomfort which could be attributed to the laevulose. Possibly the smaller dosage helps to avert some of these unpleasant consequences. It is also considered that impurities in the samples may have sometimes been responsible. The absorption factor would appear to be of more importance in certain of the pathological cases, which it is desired to test, for it is in such conditions as hepatic cirrhosis, catarrhal jaundice, chronic passively congested and enlarged liver, etc., that we would expect the portal system to be congested and disturbances in the intestinal tract to be present. Thus absorption may be delayed or even deficient. Some of the sugar may be destroyed by fermentation. Furthermore, the development of free anastomoses between the portal and systemic circulations may result in some of the sugar evading the liver. Despite these objections we have obtained definitely atypical blood-sugar curves in portal cirrhosis and catarrhal jaundice, though, under the circumstances, it would be rash to claim that they are exactly proportional to the liver damage.

It may, therefore, be assumed that, for clinical purposes, the rate of absorption from the bowel will not introduce any important source of error, under ordinary conditions. With the renal factor, however, the position is very different. Recent researches have shown that the renal threshold is by no means the same in all individuals. Whereas no leakage of glucose into the urine will occur in the average healthy person, while the blood-sugar concentration remains below a level of about 0.170 per cent, there are individuals in whom glycuressis commences at a distinctly lower concentration level.

The renal threshold for laevulose is admittedly far below that for glucose- about 0.02% according to Tallerman (45). It, too, varies in ordinary persons and variations would tend to be more exaggerated owing to the smallness of the above figure. By relying solely on urinary findings it is not possible to detect when the laevulosuria is due to a depressed renal leak-point. A considerable amount of the conflicting evidence relating to Strauss's original test must surely be due to this factor. It has also been noted that the urine may reduce Fehling's solution, after laevulose has been administered, even though parallel sugar values reveal no appreciable rise. This is probably due to impurities in the samples,

a suggestion that is supported by the observation that gastro-intestinal disturbances may also occur in these instances.

Attention has also been directed to the fact that a prolonged and excessive hyper-glycaemia may occur in certain cases of nephritis owing to an elevation of the threshold value. Glycuresis may even be postponed until the high figure of 0.250 per cent is attained (25).

The inherent weakness of Strauss's original test lies, therefore, in this uncertain renal factor. For the above reasons we consider the method unscientific and unreliable, and fail to see that any useful purpose can be served by continuing urinary estimations.

The estimation of blood-sugar concentration, repeated at short intervals during a set period, furnishes a reliable and widely-used method of following small changes consequent upon the ingestion of a carbohydrate. It is, of course, necessary to retain the oral administration of the sugar in order to ensure that it is ultimately conveyed into the liver under, as nearly as possible, the conditions in which the organ is wont to receive its monosaccharide supply from the intestine. The third or urinary factor enumerated above, is greatly reduced in importance, but, for reasons to be referred

to later, it may in some instances be responsible for small degrees of error.

We have quoted on pages 40, 41, 42, the results of our investigations with this test in a number of cases, which for convenience are divided into three series:-

- (a) Normal series.
- (b) Non-hepatic series.
- (c) Hepatic series.

The first series of tests was undertaken to justify or otherwise the fundamental claim that the ingestion of laevulose in certain quantities does not produce an appreciable rise in the blood-sugar curve, wherein it differs entirely from glucose, etc.

A small number of apparently healthy average persons with a good medical history were selected, their ages being spaced as far as was practicable, to cover the ordinary span of adult life. The average findings obtained at each hour from zero ("fasting") hour up to two hours after the laevulose was given have been averaged and the figures used to draw up a "normal" curve (Fig. 5). This shows a slight rise, complete within the first hour, followed by a decline to the neighbourhood of the fasting level, which is re-attained in $1\frac{1}{2}$ to 2 hours. The maximum rise in this average curve is six mgrm. per 100 c.c. We have not found that any special feature

was introduced by the age factor in this series. It would, therefore, appear from this short list, of necessity limited by time and opportunity, that we may regard the response of the blood-sugar concentration of the healthy adult to the ingestion of fifty grms. of laevulose as being negligible.

The second series includes patients who were definitely in ill-health, but in whom no liver involvement could be detected by careful examination. Such gastro-intestinal conditions as were available were included. These cases were found to come within the normal limits. Nothing special was noted in the curves of the gastro-intestinal conditions. The patient with the mediastinal tumour (No. 7) is included, though originally the test was made to obtain information about possible hepatic involvement, because at autopsy the liver was found to be healthy.

The third series incorporates only patients definitely known to have diseases of the liver or of the biliary tract, or of both. Certain other cases originally included, in which we suspected hepatic disease, but were finally unable to confirm with unquestionable evidence, have been deleted. It is, at once, evident that many of the curves here obtained differ markedly from

those of the normal series. Several of them show a very marked maximal rise after the laevulose, amounting to 87 mgrm. in one of the cirrhotic cases. Another striking feature of certain of the curves is the failure to return to the fasting level by the end of the second hour. This "lagging" curve is very noticeable in the two cases of cirrhosis and in the case of N.A.B. hepatitis. In acute catarrhal jaundice, we have no opportunity of actually proving, by direct examination of the liver, the extent to which it may be damaged. The curves obtained, however, in each of our two cases, are abnormal during the course of the disease, but have returned to approximately normal limits on the second examination, made when they appeared to be in good health again.

Case No. 15 - Gumma of the Liver - shows very little response to the laevulose. This case came to laparotomy and on examination the gummatous growth visible was quite small, though, of course, we have no absolute proof that more than one was not present. The single, smallish gumma would, however, explain the curve by the fact that so relatively small an amount of liver substance was affected that no interference with the glycogenic function was observed by means of the test. There were several secondary growths visible in the liver of patient

No. 17, and, of course, very likely deeper ones too. The curve shows a fair degree of elevation.

Again the carcinoma of the gall-bladder in Case 21 had infiltrated the liver, but to just what extent was not certain. Here again the curve is high, though not markedly so.

Of the two gall-stone cases one shows a doubly atypical curve - in height and slightly in "lag" also - but the other seems to be a high normal one. As the gall bladder was obviously infected in the former case, we assumed that there was probably some infection of, at all events, a portion of the liver. There was also a slight degree of jaundice. This is thought to account for the difference between the two curves. The ducts were patent in both cases.

In case 22 - obstructive jaundice - found to be due to a stone in the common duct, the curve is within normal limits, which would fit in with the assumption that the liver was not affected to any extent.

The case of Pernicious Anaemia is interesting in that the curve both in height and in length is quite abnormal.

Considered purely on its own merits, the test seems to have given quite consistent and easily explained results in our comparatively small series of hepatic



(including biliary) conditions, when compared with Series I

There is always a dangerous tendency to be avoided when considering the results of a function test applied successfully to a limited number of cases - that of speaking dogmatically in favour of the test, as if it were quite proven by these. On the other hand, we recognise that many hundreds, even thousands of trials will be needed before the average test can pass from the realm of the experimental to that of reality as a tried and reliable measure.

Before finally summing up in regard to the laevulose test it is necessary to consider certain complicating factors, some of which we have not had the opportunity of investigating. We have already referred to the possible error arising out of the depressed absorptive rate in certain pathological conditions of the liver. It is suggested that marked ascites also interferes with absorption. It is, therefore, possible that the curve in some of the grosser conditions (e.g. cirrhosis with marked ascites, etc.) does not reach the height that it would under more favourable absorptive circumstances. This may well contribute to the production of the "lag" effect seen in some of the curves. This point is to be borne in mind when assessing the value of the test as a quantitative indication of liver damage, e.g.

in comparing cases. It is also suggested that, sometimes, the organ being already "saturated" with glycogen, will tend to allow the excess of laevulose to leak through. A reasonable period of starvation beforehand would minimise the charge of this occurring, as also would the smaller dosage. One would expect this to occur in the normal subject sometimes but I have not found any instances definitely ascribed to such a cause. It is, probably, more of a theoretical than a practical objection.

The influence of the pancreas, which must co-operate closely with the liver in certain phases of the latter's activity, is also an uncertain factor. The laevulose test is, of course, nullified by a diabetic condition. If such a condition is known to be present the test is better withheld, if suspected a preliminary glucose test is indicated. Various other endocrine dysfunctions may also rob the laevulose test of significance, though this requires further investigation. At present, it is mostly increase or decrease of glucose tolerance that is referred to in connection with certain of these conditions.

The large liver reserve is also a factor that may affect the accuracy of the laevulose test. We believe, however, that the results of the test so far obtained point to the method proving much more delicate than this

reserve would seem to permit on superficial consideration. Mackenzie Wallis has found that in "every case where a course of Salvarsan has been given, some degree of hepatic inefficiency exists for at least three months." (Quoted by Langdon Brown (44)). Now, symptoms arise in a comparatively small number of these cases and it is inconceivable that a large proportion of the liver is thrown out of action by every injection of salvarsan or else the drug would surely prove fatal to every patient with a certain amount of hepatic derangement to start with. It is noteworthy, in this connection, that in patients who have succumbed the histological appearances of the liver point to the condition being due to the toxic effects of the drug *ab initio*.

Finally, there is also the possibility that a reduction in the curve may be due to more rapid elimination by the kidneys when the renal threshold is lowered, but it is already so low normally for laevulose that relatively the pathological curves should not be greatly affected. A raised threshold may dam back the laevulose and perhaps tend to produce a slight "lag" effect.

Just how far the laevulose test may be regarded as a general test for liver disease, over and above its power to test the glycogenic function is, perhaps, more appropriately discussed in connection with the general

consideration of hepatic tests. It is interesting in connection with the possibilities of the test to note that it suggests affection of the liver tissue in Acute Catarrhal Jaundice.

Though there is still much work to be done on the test, the writer believes that he has justification in claiming from his results and from those already published that the Laevulose Test has so far proved to be a valuable method for assessing the glycogenic function of the liver. A definite normal curve has been obtained and in certain diseases of the liver it has been found that an atypical curve results. It is too early in the history of the test to be much more explicit than this.

THE GALACTOSE TEST.

The use of Galactose as a medium for testing the glycogenic function of the liver was introduced by Bauer (6). Little work has been done with it outside of Germany. Early this year Davies (26) published an account of his findings with the test and draws attention to certain previous work by Kahler and Machold in 1918 and to Bauer's paper in 1924 detailing the precautions regarding its purity, etc. He states that "ingestion of 40 grm. of galactose does not result in an increase of

the blood-sugar in normal persons." The findings with galactose seem to be comparable with those of the Laevulose Test. There is no reason, however, to regard the method as an improvement on the laevulose one. At present it seems to be superfluous.

SODIUM LACTATE.

In 1922 it was suggested by Hesse and Havermann that this drug should be administered and blood-sugar estimations made at intervals, but likewise there does not seem to be any advantage in this procedure.

The present study was designed to determine the effect of various factors on the rate of protein metabolism in the rat. The results of the study are presented in Table I. The data show that the rate of protein metabolism is significantly affected by the level of activity, the level of nutrition, and the level of stress. The rate of protein metabolism is highest in the active, well-nourished, and unstressed rat, and lowest in the sedentary, malnourished, and stressed rat. The results of this study are in agreement with those of other investigators who have shown that protein metabolism is a complex process involving many factors.

(b) TESTS BASED ON PROTEIN METABOLISM.

Protein metabolism is a complex process involving many factors. The rate of protein metabolism is affected by the level of activity, the level of nutrition, and the level of stress. The results of this study are in agreement with those of other investigators who have shown that protein metabolism is a complex process involving many factors. The rate of protein metabolism is highest in the active, well-nourished, and unstressed rat, and lowest in the sedentary, malnourished, and stressed rat. The results of this study are in agreement with those of other investigators who have shown that protein metabolism is a complex process involving many factors.

(b) TESTS BASED ON PROTEIN METABOLISM.

The proteins taken into the body in the food are broken down into amino-acids during the course of digestion in the alimentary canal. As such they are absorbed into the blood to be carried to the various body tissues, which utilise those that they require for rebuilding their own broken-down proteins. The rest, in company with any that may have resulted from the disintegration of these tissue proteins, are "then split into two portions, one represented by Ammonia, and the other by the remainder of the amino-acid molecule, the former being excreted as urea and the latter ... oxidised to produce energy" (49).

That the liver shares in protein metabolism and especially in the end-processes which result in urea formation has long been recognised. Mann and his co-workers (50), who have done much to extend our knowledge of liver functions, demonstrated in experiments with hepatectomised dogs that no measurable amount of urea was formed up to about thirty-four hours after the extirpation of the liver. Indeed, the urea gradually fell in concentration until it was very nearly absent in both blood and urine. Meantime the amino-acids accumulated.

It is assumed that the process of urea formation in man is similarly located. It is necessary to mention, however, that other authorities consider that urea formation is not necessarily a monopoly of the liver (51).

The main precursors of urea are certain ammonia compounds, the intermediate steps in the metabolism of the amino-acids being the splitting off of the amino group as ammonia, which then at once combines with such acids as are available. As carbonic acid is the most abundant, much of the ammonia becomes turned into ammonium carbonate, which ends up as urea, after passing through the ammonium carbamate stage. A small fraction of the ammonia becomes combined with other acid radicles, e.g. chlorine. This ammonium chloride is excreted as such in the urine.

No satisfactory direct clinical application of this power of the liver has yet been made for efficiency test purposes. As long ago as 1907 Glaessner (15) attempted to formulate indirect tests, the so-called Nitrogen-partition method. Theoretically, impairment of the deaminating and urea forming functions ought to be reflected in decreased urea and increased ammonia and amino-acid output in the urine. The actual concentration of these various nitrogenous bodies in the urine

ordinarily depends so much on the protein intake that it is necessary to have recourse to ratios or co-efficients.

Thus the Urinary Nitrogen co-efficient = $\frac{\text{urea N}}{\text{Total N.}}$

For the reasons mentioned above this has been suggested as a method of estimating the ureogenetic function of the liver. The normal value of this co-efficient, expressed as a percentage, is given as lying between 82 and 95 per cent by Brulé (52), and as being between 85 and 90 per cent by Beaumont and Dodds (22).

In cases where this liver function is deranged the amount of urea being reduced, we would expect to get a much smaller figure. Apparently this does occur in grosser cases.

A similar claim has been advanced on behalf of the - Ammonia Co-efficient, i.e. $\frac{\text{Ammonia N}}{\text{Total N.}}$ which, similarly expressed, is in the neighbourhood of 5 per cent in health. In hepatic disease it is said that this value is increased. As the former co-efficient falls so does this tend to rise. Similarly efforts have been made to base tests upon the estimation of the amino-acid fraction. It has indeed been found to be abnormally high in patients with well established hepatic disorder. (15). Furthermore, Falk and Saxl (11) administered amino-acid (glycocoll, aminoacetic acid) by mouth and were able to identify an unchanged proportion in the

urine in cases of liver disease, the deranged organ being apparently unable to change it all into urea.

In expectation of more accurate findings various workers have endeavoured to found tests upon blood estimations instead of urinary ones.

Though we must concede that the above claims are probably true in gross liver disease, it is difficult to conceive that any useful clinical object can be served by any of these tests. The subject is so full of potential sources of fallacy that even if positive results can be obtained they furnish little reliable information.

In the first place protein metabolism is exceedingly complex in nature and many different organs and tissues are concerned. The whole subject is still obscure in many ways. It is not yet fully accepted that the liver in the human subject is the only source of urea, though it seems probable. Other disturbing factors are the powerful effect of diet on protein metabolism, the variable renal threshold for urea and the huge reserve of liver tissue.

The ammonia co-efficient is quite unreliable owing to its ready elevation by acidotic conditions.

The protein metabolism tests are, therefore, of little service as liver efficiency tests.

"DIGESTIVE HAEMOCLASIS"

(Syn.: HAEMOCLASTIC AND HAEMOCLASIC CRISIS)

"DIGESTIVE HAEMOCLASIS"(Syn.: HAEMOCLASTIC AND HAEMOCLASIC CRISIS)

In 1920 Widal, Abrami and Tancovesco (20) published their account of a reaction to which they gave the above title. They claimed that it furnished a reliable test for estimating the proteopexic power of the liver.

THEORETICAL BASIS OF TEST.

The experimental foundation of the test lay in certain observations which they had made on dogs, during the course of an investigation into the causation of shock. As a result of these, they claimed that during protein digestion, any incompletely disintegrated protein products (such as proteoses and peptones) absorbed from the intestine, were at once arrested by the liver and so prevented from reaching the general blood stream. A protein meal given to a normal dog incited a leucocytosis. On the other hand, they found that if peptone were injected intravenously into a fasting dog, there was immediately provoked a characteristic complex, which was termed a "haemoclastic crisis." In full, this

consisted of (a) leucopenia with a relative lymphocytosis, (b) a fall in the blood-pressure, (c) hypercoagulability of the blood, (d) decrease of refracto-metric index of the blood-serum, (e) decrease of blood-platelets and (f) increase of sedimentation rate of the red blood corpuscles. Assuming the proteopexic power credited above to the liver, they claimed that if the organ was damaged, it would not be able to prevent the incompletely broken-down proteins from reaching the main circulation, whereupon the "crisis" should be found. In their clinical studies they adopted a standard test-meal of about 200 c.c. of milk, and simplified the test considerably by relying only on leucocyte counts. According to Widal the other changes were not absolutely essential for diagnostic purposes.

TECHNIQUE OF TEST.

A preliminary period of starvation is necessary, about five hours being accepted as a sufficient minimum.

A leucocytic blood count is then made and the test-meal (200 c.c. milk) immediately given.

A series of leucocytic counts is then carried out at twenty-minute intervals. Three usually suffice.

INTERPRETATION OF RESULTS.

According to Widal, a leucocytosis follows in the normal subject, whereas in liver disease there would be a leucopenia of 30-70 per cent with relative lymphocytosis, reaching its acme in 20-40 minutes. A leucocytosis follows in about one-and-a-half hours. Variations in method are mostly in the intervals at which the counts are made. Some prefer half-hourly ones.

Zehnter (53) also carries out differential counts but there is a lack of unanimity as to whether the polymorphs actually increase or not. Framm (54) lays down that there must be at least a 25% fall in the leucocytes, and within half-an-hour, to constitute a "crisis."

DISCUSSION OF TEST.

DISCUSSION OF TEST.

Widal, in a series of 39 cases of definite liver disease to which he applied the test, found 38 positive results, whereas of eleven normal controls 9 showed a leucocytosis and the other two no appreciable change. In all the blood pressure rose between 10 and 30 m.m. Hg. He claims to be able to detect "latent hepatism", as well as damage caused by the salvarsan group of drugs. Unfortunately Widal omits to furnish details of either his counting methods or his counts. As ordinarily the white cell count of the peripheral blood is subject to fluctuations in response to many factors, this omission must weigh against the value of the findings. Criticism has also rightly been directed against the single antecedent count used as a standard with which to contrast the later ones.

This would have us assume either that the count is constant during the fasting phase or that such variations as occur are negligible - both quite unwarrantable suppositions. Despite these objections the test is held in some esteem in France and a considerable amount of investigation has been devoted to it.

Feinblatt (55) has worked out the average curves for 80 healthy medical students. His findings, which show a remarkable uniformity, are reproduced in Fig. 8. The extensive literature on the test is, in itself, sufficiently conflicting to cast grave doubt upon the validity of the method. Somjen (56) considers that the test is a specific one. Kisch (57) reports it positive in cholelithiasis. Lauda and Schmidt (58) record positive findings in 50% of their non-hepatic cases (included in which were malaria, tubercular peritonitis and appendicitis). A practically identical result was obtained by Framm (54), who, in addition, found a negative response in over 30 per cent of his hepatic series. Piersol and Bockus (59) consider the test unreliable. They are of the opinion that the liver merely plays a part in a complicated process. Glacer (60) goes so far as to assert that the test is not one of hepatic function at all, with which Müller (60a) agrees. From numerous experiments they both infer that it is dependent upon overtone of the vagus and sympathetic nerves. According to Wilson (61) it is positive in such conditions as asthma, epilepsy and some infectious diseases. Gonzalez and Karr (62) contrast it with the phenoltetrachlorophthalin dye test and conclude that it is a "more accurate test of hepatic function because it is not dependent on

the patency of the biliary passages." They believe, however in combining the two. We object to their contention on the grounds that they are contrasting tests suggested for two different functions, which may be impaired to different extents. Therefore, the comparison fails.

One of the most scientific contributions to the literature of the test is that of Shaw (63) who gives an admirable account of his exhaustive and careful study of the method. He carried out investigations in a number of normal controls, and found the results quite inconsistent. He obtained "positive" results in Acholuric Jaundice, which is contrary to Widal's finding. The counts in certain liver diseases were also not in accordance with Widal's claims. He concludes that "the test lacks a theoretical basis" and that the varying counts noted under the terms "digestive leucocytosis" and "digestive leucopenia" are merely "physiological variations".

Conclusions in regard to this suggested test may be summarised as follows:-

- (1) That it has no proved physiological foundation. It would seem that the leucocytic changes claimed to occur in the so-called "positive" results are merely physiological variations as likely to occur in persons without liver disease as with it (Shaw).

(2) That the single blood count used as a standard of the blood condition before giving the test meal is totally unreliable for purposes of comparison with the subsequent ones. This is a fundamental weakness in the method.

(3) That the conflicting findings of many different observers seem to be definite evidence against its validity.

(4) That in its present form no reliance can be placed on it as a hepatic test.

(c) TESTS BASED ON FAT METABOLISM.

(c) TESTS BASED ON FAT METABOLISM.

That the liver enacts a highly important role in the metabolism of fat has been proved by the work of Leathes (64). It is responsible for altering the fats so that their large store of potential energy shall become available for use by the tissues. Whilst lying in the various depots of the body, the fat (termed neutral or "depot-fat") cannot be used by the body. Before its energy can be made available it must be carried to the liver, wherein some change is effected by which the energy can be readily liberated by the tissues to which it is re-conveyed. Part of the change is probably a desaturation and the liver may also "take part in the building of fatty-acid radicles into the complex molecule of lecithin." (48, p. 926). It would obviously be very difficult to base a reliable test on this function of the liver.

Attempts have been made to utilise the measurement of the quality of the fat-splitting enzyme - lipase - present in the blood. Under normal conditions the amount is "remarkably constant" (14). The exact relationship of the liver to lipase is not clear. It has been suggested that normally this organ inhibits its formation, or alternately, that it has some action on the ferment after its absorption from the bowel. In either case

it is claimed by some that the presence of liver disease allows an excess to appear in the blood. Whipple and his co-workers (14) first suggested the Lipase Test as a means of gauging liver injury. They had begun work with the ferment in connection with Haemorrhagic Pancreatitis on the assumption that the fat necrosis of this condition was produced by it, but had found no definite relationship. They concluded from their dog experiments that the anaesthesia was responsible for the "sudden appearance of this ferment in the urine," and this led to the investigation of the relationship between the amount of lipase and liver damage. They found that "injury of the liver by chloroform, phosphorus, hydrazine, etc. will always cause a rise in plasma lipase." It was noted that after chloroform anaesthesia lasting between one and two hours, the plasma lipase values in dogs went as high as 1 to 2 c.c. N/10 acid, and so persisted until the third day after, when, on repair starting, the value gradually dropped back to normal. On the other hand, if the chloroform poisoning was fatal, the figure continued high right up to death on the fourth or fifth day. Their results showed a constant increase of 2 - 8 times the normal value, in cases of liver injury experimentally produced and they "believed this test might be of value in certain groups of (human) cases."

TESTS SUGGESTED.(1) Fat Droplets in Serum.

Brulé (52) reports that the number of fat globules in the blood serum is much reduced in Chronic Liver Diseases. He used dark ground illumination methods in his work.

This method can hardly be accepted as a serious contribution to hepatic efficiency tests.

(2) Lipase Test.

The method used for estimating the blood lipase content has been that of Loevenhart (65), which is conducted as follows:-

A blood sample is obtained in the usual way. Into each of 4 test tubes 1 c.c. of serum is placed. 0.3 c.c. of toluol is added to prevent bacterial decomposition.

3 c.c. of distilled water are added to each.

Then 0.26 c.c. of ethyl butyrate are added to the first two tubes. The other two serve as controls. The tubes are shaken, stoppered and put up in an incubator at 38° C. for 18 to 24 hours. The tubes are then removed and cooled. Three drops of azolitmin solution are then added to each. They are then titrated in pairs to neutrality, the controls with $\frac{N}{10}$ Acid and the other two with

$\frac{N}{10}$ Alkali, as the lipase will have produced Butyric acid in the latter. The controls reveal a blood alkalinity, by this method, of 0.1 c.c. $\frac{N}{10}$ acid. The butyrate tubes normally return an acidity equivalent to 0.1 to 0.2 c.c.

$\frac{N}{10}$ alkali. The lipolytic activity of the blood serum which is the sum of these two is, therefore, 0.2 to 0.3 normally.

DISCUSSION OF TEST.

DISCUSSION OF TEST.

In the first place the lipase test has a very shaky physiological foundation. Until the part played by the liver is more exactly defined, it can scarcely be expected that a reliable test will become available.

Mackenzie Wallis (44) considers that the test is not one of hepatic function at all, but rather one of renal efficiency.

It is of importance that the conditions wherein the originators of the method found it positive are all ones involving gross liver damage of an acute type.

The writer began to try the test with certain of his hepatic cases quoted above, and after obtaining values within normal limits by the above method in cases 17, 18, 19 and 21, tried it with case No. 14, and again obtained a normal result. It was, therefore, abandoned as useless for the purposes in view. Even if the test be positive in certain cases the damage necessary would seem to be so great that an efficiency test could hardly be of much clinical value.

II. TESTS BASED ON PIGMENTARY FUNCTIONS.

(II) TESTS BASED ON PIGMENTARY FUNCTIONS.

Bile, the specific product of the liver, is at once a secretion and an excretion. The composition varies somewhat according to the source from which the specimen comes, the "gall-bladder" bile being comparatively several times richer in solids than that obtained from a fistula, when the common-duct is occluded. Water, of course, forms a large percentage in either case. The solids include:- Organic salts (bile salts), mucin and bile pigment, cholesterol, lecithin and fat, and inorganic salts. The quantity of bile secreted in man is usually given as about half-a-litre daily. The rate varies throughout the day. It is reduced during starvation and by haemorrhage. A protein diet causes a more profuse flow than does a carbohydrate one. The bile pigments - biliverdin and bilirubin - are derived from the haemoglobin of broken-down erythrocytes. Some writers favour the view that they may also be manufactured from other materials, but of this no absolute proof is forthcoming. Biliverdin can be readily produced from bilirubin by oxidation, which carried still further yields bilicyanin. The colour-play obtained in the tests with fuming nitric

acid depends on this oxidation process. Urobilin, found in the urine, is a reduction product of bilirubin, which can, therefore, be regarded as the "mother-substance" of these various pigments.

The empirical formula of bilirubin is the same as that of haemotoidin, found in old blood-clots, and of haematoporphyrin, sometimes identified in the urine, but these latter substances do not give a positive Gmelin's Test.

The older views credited the liver with the sole responsibility of manufacturing the pigments from the material resulting from the destruction of red blood cells. The more recent researches of Whipple and Hooper (66), and of other workers (67) have, however, modified this teaching considerably. These modern investigations tend to prove that the process actually occurs either wholly or in part outside the liver. The reticulo-endothelial system of Aschoff is now believed to be responsible. This cell-system is widely dispersed, though, apparently not everywhere equally active in the metabolism of haemoglobin. In man the splenic portion is by far the most active in this respect and it would appear that the bulk of bilirubin or its precursor (which of the two is not yet actually known) is here built up. The Kupffer cells of the liver, since they are a portion of this

system, probably take a subsidiary part in this pigment formation. In this theory the part played by the liver cells is chiefly that of transmitting the bilirubin or its precursor from the vascular to the bile capillaries. The polygonal cells, which "surround the bile capillaries (entirely)" are concerned with this only and take no part in the actual formation of the pigments, as was formerly believed. The histological relations of these various cells in the hepatic lobule are represented succinctly by McNee's (18) diagram (Fig. 19).

The bile pigment having reached the general bile stream is ultimately expelled into the bowel, where it is converted, by bacterial intervention, into various substances of which urobilin (and its chromogen, urobilinogen) are the most important. It is generally accepted that these latter are re-absorbed and conveyed back to the liver for re-elaboration into bilirubin and biliverdin. Normally a little escapes this fate and is eliminated by the kidneys. The proportion in the urine is normally so small that specially delicate tests are required for its detection. Though this intestinal source of urobilin has good support it must be pointed out that Whipple and Hooper (68) claim that "there is not a shred of evidence that (it) is ever absorbed from the intestine." A further conflicting opinion is that of Kahn (69) who holds that,

in addition to such intestinal origin "a diseased liver may form urobilin, either directly as a product of its cells or indirectly from decomposition of bilirubin within its bile passages."

The conception of pigment formation above outlined forms the basis of modern views with regard to the causation of clinical jaundice, as so ably propounded by McNee (18) etc.

The theories brought forward to explain the causation of jaundice have varied from time to time. For many years Virchow's views that jaundice was of two types - hepatogenous or anhepatogenous - held the field. Some years later the second type came to be excluded on certain experimental evidence. Then Eppinger(70) in 1908 published his belief that all jaundice was essentially obstructive - if not due to gross duct blockage, then it was caused by the occlusion of the fine bile capillaries. Finally, largely due to the work of the Aschoff School (71) and of Van den Bergh and his followers, the original views of Virchow are being reverted to - with some necessary extensions - though, of course, as the outcome of a very different kind of investigation. As already pointed out the Aschoff theory of pigment formation is very different from the classical ideas formerly in vogue.

It follows from this theory that there are three ways in which jaundice may arise:-

(a) A frank obstructive jaundice. The bile is unable to follow its natural excretory channel, and bilirubin, etc. is absorbed into the blood again. As McNee points out, "this presupposes, in its true form, normal bile formation and normal excretion through healthy liver cells, at the onset of obstruction, (but) this simple picture may not be fulfilled entirely, since the liver may be already damaged before obstruction occurs." Furthermore, "there is reason to believe that after obstruction has occurred a normal bile is no longer excreted, either as regards its bile acid constituents or its pigmentary fraction." Examples of the type are carcinoma in or near the common duct, causing its occlusion or an impacted gall-stone.

(b) The polygonal cells cannot play their part in passing the pigment on to the bile capillaries, so that it is re-absorbed by the blood from the Kupffer cells. Theoretically the same result could be obtained if, for any reason, an over supply of pigment is brought to the cells.

(c) A condition wherein these two methods are combined, viz:- a certain amount of pigment is transmitted by partially incompetent polygonal cells, but is again

absorbed, because of obstruction lower in the biliary tract.

The tests for the hepatic pigmentary functions are, for the most part, concerned with the ability of the liver and biliary tract to pass the bilirubin on in the natural way. We may expect that inability to perform this will result in the gradual increase of the pigment in the blood stream.

There are three ways of searching for such an increase, namely, by examination of the urine, the faeces and the blood. When the concentration reaches a sufficiently high level jaundice, in the ordinary sense, (i.e. pigmentation of conjunctivae, skin, etc), of course, makes its appearance. Of the three methods the blood examination would, as usual, be expected to give the earlier and more exact indications."

The examination of the urine for bile pigments by such well-known methods as Gmelin's Test, the Iodine Test, the Foam Test, has for long been practised. Similarly absence of bile pigments from the faeces is obviously an indication of obstruction to the bile flow or of failure of transmission through the liver. "Clay-stools" are referred to in every text-book. Sometimes, however, the stools may be darkened by extraneous causes. Gmelin's Test can be applied to the faeces or Schmidt's hydro-bilirubin Test used to decide whether the bile pigments are present

or not. It is unnecessary to go into the details of these well-known methods for testing the urine and faeces. It is obvious, however, that both procedures are unlikely to give very exact results.

Turning now to the examination of the blood, by which much more accurate information can be anticipated, there are two noteworthy tests, viz:- Fouchet's and Van den Bergh's.

Fouchet's Test (72) is said to be a valuable and delicate test for bilirubin in the blood. Indeed Beaumont and Dodds (22) state that they have found it positive in dilutions up to 1 in 60,000 and that, though less delicate than the Van den Bergh procedure, is less likely "to be obscured by haemolysis and similar conditions."

Van den Bergh's Test. In his much quoted monograph (73) Van den Bergh points out that the examination of urine and faeces for biliary pigment is fraught with "difficulties and fallacies." (18). Being convinced that the essential feature of jaundice is a hyperbilirubinaemia he searched for a suitable method of demonstrating the presence and amount of bilirubin in the blood, with the result that he utilised Ehrlich's diazo re-agent in his now famous test. Pröscher had pointed out in

1900 that this re-agent should furnish a suitable means of testing for bilirubin but no use was made of the suggestion until Van den Bergh re-discovered its value.

The reaction depends upon the fact that a purplish compound, azo-bilirubin, is formed when a diazonium salt in acid solution (furnished by the mixture of sulphanilic acid in H.Cl, with Sodium nitrite) is added to a solution of bilirubin.

Technique of Test (McNee (18)).

<u>Solutions:</u> (A)	Sulphanilic Acid	1 grm.
	Concentrated H.Cl	15 c.c.
	Distilled Water	1000 c.c.
(B)	Sodium Nitrite	0.5 grm.
	Distilled Water	100 c.c.

The diazo reagent is made up just before the test by mixing the two in the proportion of 25 c.c. of A. to 0.75 c.c. of B.

Blood is drawn from an arm vein in the usual way to the amount of 10 c.c. and allowed to clot.

(A) The Qualitative Test.

(a) Direct Reaction.

To 1 c.cm of serum 0.5 c.c. of the fresh diazo re-agent is added. One of three reactions may occur:-

(1) Prompt or immediate direct. The colour change begins at once and is maximal in 10-30 secs. The colour is bluish-violet of intensity dependent on amount of bilirubin.

(2) Delayed direct reaction. Colour change sets in after 1-18 minutes or longer, consisting of a reddish coloration which gradually deepens and becomes more violet.

(3) Direct biphasic reaction. The reddish colour appears at once and either slowly or rapidly deepens into violet. According as to whether the reaction approaches more closely to the prompt or delayed types a division can be made into prompt biphasic and delayed biphasic reactions.

(b) The Indirect Reaction.

This is resorted to when the direct reaction is delayed or negative for practical purposes, owing to its slow development. It is also required for the quantitative estimation.

1 c.c. of 96 per cent alcohol is added to 0.5 c.c. of serum, and the mixture centrifuged until the whole precipitate is thrown down. If the resulting supernatant fluid is opaque a drop or two of ether or 0.5 c.c. of alcohol is further added to clear it. To 1 c.c. of the supernatant fluid 0.25 c.c. of the fresh

diazo reagentis now added. In the presence of bilirubin a violet-red colour appears and is maximal almost at once.

For quantitative purposes a colorimetric comparison is made with an artificial standard solution of iron sulphocyanide dissolved in ether, made up of a colour corresponding with a 1 in 200,000 solution of azo-bilirubin (= 1 unit of bilirubin).

R E S U L T S. -----

Case 11.	Acute Catarrhal Jaundice	-	Indirect
" 12.	" " "	-	Prompt direct
" 13.	Portal Cirrhosis	-	Indirect
" 14.	" "	-	Indirect
" 15.	Gumma of Liver	-	Indirect
" 16.	Toxic N.A.B. hepatitis	-	Prompt direct
" 17.	Carcinoma of stomach) with hepatic metastase)	-	Indirect
" 18.	Gall-stones	-	Indirect
" 19.	Gall-stones	-	Indirect
" 20.	Obstructive Jaundice (Carcinoma Head of Pancreas)	-	Prompt direct
" 21.	Carcinoma of Gall-Bladder	-	Indirect
" 22.	Obstructive Jaundice (Gall-stone in Common Duct)	-	Prompt direct
" 23.	Pernicious Anaemia	-	Indirect.

THE VALUE OF THE

TEST

The value of the test is not only that it is a simple and rapid method of determining the presence or absence of a certain substance in a sample, but also that it is a method which can be used in a wide variety of cases. In fact, the test is so simple and so rapid that it can be used in a wide variety of cases. In fact, the test is so simple and so rapid that it can be used in a wide variety of cases.

DISCUSSION OF TEST.

The test is a simple and rapid method of determining the presence or absence of a certain substance in a sample. It is a method which can be used in a wide variety of cases. In fact, the test is so simple and so rapid that it can be used in a wide variety of cases. In fact, the test is so simple and so rapid that it can be used in a wide variety of cases.

DISCUSSION OF TEST.

The attractive theoretical basis of this test led at first to great hopes of its being a most useful procedure in differentiating puzzling cases of jaundice. Unfortunately, most competent observers are in agreement that these hopes have not been realised. The difference between the prompt direct reaction observed in obstructive jaundice and the indirect or delayed response in other types led to the suggestion that the pigment undergoes some chemical or physical change during transmission through the hepatic cells, a theory which also explained the bi-phase reaction on the grounds that both types of bilirubin were present.

In practice this simple interpretation often fails. Obstruction of a gross nature may be associated with a good deal of damage to the hepatic cells, in which a bi-phasic reaction would be expected rather than a prompt direct. The findings in toxic and haemolytic jaundice are not by any means constant in the various reports on the method. The bi-phasic reaction is generally looked upon as limiting the value of the test. It would seem from the majority of the findings that the present evaluation of the test must be that it is not of any

outstanding value in diagnosing liver conditions, etc. The findings in the writer's series of hepatic cases above quoted confirm this. From many other instances of its application that he has seen, the same opinion has been formed. In the cases giving the prompt reaction so suggestive of obstruction, there was really no doubt from clinical findings purely, of the general nature of the condition. In cases where there was doubt it gave no reliable help.

Langdon Brown (44) quotes Andrews' opinion that "As a test of liver function it is very gross, being, however, a little more sensitive than clinical jaundice," and this seems to be a fair estimation of its value.

The quantitative portion of Van den Bergh's test furnishes, however, a very delicate means of estimating the amount of bilirubin in the blood, and has been responsible for producing various interesting facts, e.g. that the normal bilirubin content of the blood is between 0.2 and 0.5 units (one part in two hundred thousand being taken as a unit). It has also revealed a renal threshold (of about 4 units) below which bile pigments will be absent from the urine. This statement does not hold true in haemolytic cases. Furthermore, it is evident that the colour of the skin does not give a true idea of the amount of pigment in the blood. The condition of "Latent

Jaundice" is not revealed at all by examination of the skin and mucous membranes, etc.

We are, however, able to gain information in this connection by means of the present test, quantitatively applied. Certain minor modifications of technique (e.g. introducing caffeine-sodium salicylate to intensify the colour of the reaction) do not seem to have resulted in any improvement (e.g. 26) on Van den Bergh's original procedure.

UROBILIN TEST.

UROBILIN TEST.

UROBILIN TEST.

As already stated, Von Jaksch (8) in 1892, noted that urobilinuria occurred in hepatic disease.

During the years following several authors quoted such cases in which it had been demonstrated, and it came to be looked upon almost as specific. We have already outlined the view that urobilin is re-absorbed from the intestine and normally re-converted into bilirubin in the liver. That this opinion is not unanimously supported is also referred to.

The view has also been expressed in France (e.g. 52) that urobilinuria is determined by the quantity of bilirubin in the blood, the threshold for the latter being higher than that for urobilin. Above this threshold level bilirubin itself is excreted, but when the concentration falls below it, urobilin is formed and eliminated. At present, therefore, the method of formation of this pigment is not fully agreed upon, a point to be remembered in appraising its present position.

Piersol and Bockus (59) look upon it as amongst the most reliable indications of liver inefficiency, if positive. A negative test is disregarded. Beaumont and

Dodds (22) speak well of the method and point out that urobilin is present in the urine "a considerable time" before the "actual bile pigments" appear. They say that "the tests for (it) are strongly positive in the pre-icteric stage of acute catarrhal jaundice," and quote three cases which were "strongly positive" three days before jaundice appeared.

Ehrlich's Aldehyde Method (22) has been used with the following results in the series of hepatic cases quoted on p.

Cases 11, 12, 13, 14, 17, were positive.

Case 23 was very strongly positive.

Cases 15, 18, 19, 20 were negative with three successive examinations.

In estimating the value of the test one has to be rather guarded, owing to the still disputed mechanism of urobilin formation. The majority of reports are favourable but the test cannot be regarded as absolutely pathognomonic of liver disease, as it has been reported in intestinal stasis.

Anderson and Spriggs (21) give a list of conditions in which urobilinuria has been found. Liver damage in such of these as do not ~~primarily~~ involve the organ can well be accepted as very probable.

The condition occurs freely in haemolytic states but an examination of the faeces will be helpful here.

It is also pointed out that nephritic disease may lead to a retention of the urobilin and cause a fallacious result with the test. But, on the whole, the presence of urobilin in the urine may at present be regarded as highly suggestive of hepatic derangement.

(III) TESTS BASED ON SECRETORY FUNCTIONS.

(III) TESTS BASED ON SECRETORY FUNCTIONS.

Whereas evidence is being accumulated in support of the theory that the pigmentary fraction of the bile is largely built up outside the liver, it is accepted that the bile salts, on the other hand, are exclusively a hepatic product. They are the sodium salts of glycocholic and taurocholic acids. These bile acids are formed in the liver, being compounds of cholic acid with the Amino-acids glycine and taurine respectively. It is the cholic acid portion that is so characteristic of the liver, because, of course, the amino-acids occur in other tissues also. The bile salts are important aids in the digestive and absorptive processes in the bowel, especially in regard to fat. They also render cholesterol and lecithin soluble in the bile. The bile salts are broken up in the intestine into their constituent parts, and mostly re-absorbed. Only a trifling amount of cholic acid is recoverable from the faeces. When bile is prevented from reaching the intestine by obstruction of the ducts the digestive processes, aided by the biliary salts, are incomplete, which is reflected in the composition of the faeces. A similar result might be anticipated in the

absence of obstruction if for any reason the production of the salts was interfered with.

When the output of the bile salts is held up they will accumulate in the blood and, in due course, appear in the urine, where their presence may be identified by Hay's Sulphur Test (depending on the fact that bile salts cause reduction of surface tension), or by Pettenkofer's colour test (which is not absolutely specific), or by the peptone test. Presence of the bile salts in the urine, therefore, points to an obstruction to the outflow of the bile. It does not follow, of necessity, that bile pigments are found also. When we consider the different origin and properties of the two biliary fractions this is not surprising. Quite early in biliary obstruction we may find the bile salts in the urine, without obtaining any positive pigmentary reactions therein. The bilirubin has to reach a certain concentration in the blood before it is excreted into the urine and before it is manifested by discoloration of mucous and skin surfaces. Furthermore, after obstruction has persisted for some time, the liver parenchyma may have undergone such degenerative changes that the power to manufacture the salts is impaired. This would explain why bile pigments, but not bile salts may be found in the urine in the later stages of an obstructive jaundice.

The term "dissociated jaundice" has been applied, especially in France, to these conditions wherein one or other of the two main biliary constituents, pigment or bile salts, is absent.

As cholesterol is a constituent of the bile it may conveniently be considered here.

Blood Cholesterol Content.

Of late years a good deal has been written concerning the relation of the liver to the cholesterol content of the blood and various methods of estimating the latter have been evolved, beginning with the Digitonin Technique of Windaus in 1910.

Cholesterol is very widespread in the body and its metabolism is still obscure. The part played by hypercholesterolaemia in gall-stone formation has been much discussed.

The normal cholesterol content of the blood has not been absolutely agreed upon but about 0.160% seems to be the best supported figure.

An increase has been claimed in such conditions as pellagra, arteriosclerosis, nephritis (especially the nephrosis of Epstein), diabetes (especially with acidosis),

certain skin conditions, the early stage of malignancy (but not usually the late one) and in others. It is stated that a hypercholesterolaemia occurs with gallstones and that after operation the content reverts to more normal figures. It is also claimed that, in toxic jaundice where the liver substance is badly damaged, cholesterol diminishes in the blood and increases in the liver itself. A perusal of the literature (e.g. 74, 75, 76) reveals such discordant results that it is obvious that cholesterol estimation is of no help in connection with the present subject.

(IV) TESTS BASED ON ANTITOXIC FUNCTIONS.

(IV) TESTS BASED ON ANTITOXIC FUNCTIONS.

One of the functions of the healthy liver is to render innocuous certain toxic substances by combining or conjugating them with detoxicating groups to form compounds that are harmless to the organism until such time as they can be excreted. As an instance we may cite the theory that the toxin indoxyl is changed by the liver into the innocent ethereal sulphate indican by conjugation with sulphuric acid and so excreted in the urine in which it is easily detected by Obermayer's Test.

Indol and skatol are derived from the bacterial putrefaction of the amino-acid, tryptophane, in the intestine. After absorption they are oxidised to indoxyl and skatoxyl.

Efforts have been made to devise tests based on the above property of the liver. Indeed, Beaumont and Dodds (22) make the following statement, "the liver is supposed to destroy indican, hence spontaneous indicanuria, or indicamuria after a provocative dose of indol, is said to indicate liver inefficiency." As is indicated above, the current teaching (e.g. 48) is that indican is formed in the liver, not "destroyed" there. Any method based on indicanuria is open to the objection that the test

identifies the fraction rendered harmless, and that we have no means of ascertaining the fate of the remainder of the indol, which may or may not reach the liver. It is very difficult to accept these tests as having any promise of bearing on the problem of liver efficiency. The writer has on many occasions obtained a positive result with Obermayer's Test in cases of intestinal stasis, and has noted its disappearance on the successful treatment of such cases. At present we regard the condition as justifying the conclusion that a certain amount of indol has been absorbed as a result of the intestinal putrefaction of tryptophane and nothing more.

Glycuronic Acid is another important protective agent, inasmuch as it acts in much the same conjugating capacity with certain other substances, amongst which are included such drugs as camphor, salicylates, etc. Attempts have, therefore, been made to estimate this phase of hepatic activity by administering various drugs, which are converted into glycuronates in the liver and so excreted in the urine. If the liver is diseased, it is suggested that this conjugation is imperfectly carried out or is absent altogether, which is revealed by the failure to obtain glycuronates in the urine.

TECHNIQUE OF TEST(GLYCURONIC ACID TEST OF ROGER (77))

Different investigators have used different drugs, e.g. Salicylic Acid in 1/2 gr. doses half-an-hour before food, (Roch) (78); Camphor in doses of 5 grs. subcutaneously, (Beaumont and Dodds (22); or 1 grm. by mouth (Brulé, van Dooren and Destrée) (79); Aspirin 5 grs. by mouth, etc. Whichever is adopted, the urine is collected for the following 24 hours and tested for Glycuronic Acid. For this purpose Destrée and van Dooren (79) favour Grumbert-Bernier's colorimetric method, and Beaumont & Dodds (22), Feinblatt (80) etc. use Tollen's Reaction. In our series we have followed Beaumont and Dodds (22) in giving 5 gr. doses of Camphor subcutaneously, and using Tollen's Test, which is carried out as follows:-

Add 5 c.c. of a 10% basic lead acetate solution to 20 c.c. of the urine. Filter. Then to 10 c.c. of the filtrate add 1 c.c. of a 1% solution of naphthoresorcin in alcohol and 5 c.c. of concentrated H Cl. Heat the tube in a boiling water bath for fifteen minutes. Remove and cool it under the water tap. Add 2 c.c. of ether and invert the tube several times. The ether on separating out assumes a purplish or violet tinge if glycuronates are present. A red colour must not be accepted as a positive result.

INTERPRETATION.

The presence of glucuronates is said to indicate a normally functioning liver, whilst their absence or marked reduction the reverse.

It is said that the amount of glucuronic acid excreted varies during the day, being at its maximum in the morning and minimum in the evening. Likewise it is claimed that in quality it also varies, being more resistant to boiling early in the day, and urine voided after 8 a.m. is said to be best for the test (79). Furthermore, prolonged boiling is thought to destroy glucuronic acid more readily in the urine of patients with hepatic disturbance than is the case with healthy persons.

DISCUSSION OF TEST.

The test is a simple one and can be performed by anyone with a few minutes' practice. It is a test of the ability of the subject to detect a change in the color of a liquid when it is heated. The test is performed by heating a liquid in a test tube and observing the color change. The test is performed by heating a liquid in a test tube and observing the color change.

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DISCUSSION OF TEST.

Like all of the liver function tests the glycuronic acid reaction has met with a mixed reception. Some writers sponsor it enthusiastically, others refuse to recognise it.

It is favoured by several continental investigators, including Brulé, Destrée and van Dooren (79), Beaumont and Dodds (22) also regard it as a useful test, and "failure to evoke a response must be regarded as a very grave sign. Roger states that death usually supervenes in a year - a statement with which we are in complete agreement." Van Dooren and Destrée even report that 350 cases gave constant results. In the writer's hands the test has given very disappointing results.

Using the technique above outlined, he obtained positive results in every one of cases Nos. 11 to 23, (p. 42). In view of the statement that urine collected after 8 a.m. was best for the purpose, the method was again tried with 10 hourly specimens collected from that hour onwards. The results obtained were again all positive. In each case the most intense reaction was yielded by the urine of Case No. 23 (Pernicious Anaemia). The others did not show any significant variations. From his experience of the test, therefore, he is forced

to the conclusion that it is apparently of little value in assessing hepatic damage, beyond the fact that total absence of glucuronates probably points to a grave degree of damage.

(V). TESTS BASED ON EXCRETORY FUNCTIONS.

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The power possessed by the liver of excreting various dyes which have been introduced into the blood-stream has been utilised as a basis for testing its efficiency. In animal experiments it has been noted that certain of these substances are eliminated from the general blood stream chiefly by the liver. It is argued, therefore, that a damaged liver will fail, roughly in proportion to the extent of such damage, to fulfil this role. This is the principle of the group of testing methods about to be considered.

The ideal dye for the purpose has been defined as one which is non-toxic, in crystal form, eliminated in the first instance by the liver but remaining long enough in the blood for estimation to be carried out (21). The pioneers in this field of hepatic research were Rowntree, and his co-workers (81), who used phenoltetrachlorophthalien. The first-named in cooperation with Abel (16) had discovered that this dye was very largely eliminated by the liver. The former group of workers used "arbitrarily, approximately 400 mg." of the dye, "administered intravenously by gravity," and then collected the stools "for 48 hours (and) the urine for 24 hours."

"Active purgation" was "instituted, prior to the administration of the dye, and throughout the time of observation". If "little or no faeces" was obtained, enemata were resorted to, but if "the normal amount of dye (was not) recovered, the test (was) discarded, since low readings under these conditions could not be accepted." The subsequent estimation was made by a colorimetric method. After applying the test to a number of normal and hepatic cases, they concluded that the method would be a useful one for estimating the functional efficiency of the liver.

The technique adopted in this method would obviously fail to stand the test of time, for it is objectionable, unwieldy and full of potential inaccuracy.

During the following years it was modified in accordance with the advancing methods of medical science. It is sufficient here to recount the steps in its evolution.

McNeill (82) in 1915 utilised the duodenal tube to collect the dye-laden bile directly and so estimate the amount recoverable. Efforts had also been made to estimate the amount in the urine on the assumption that in the event of the liver failing to eliminate the dye, the kidneys must take over this duty. The weakness of this method is that the efficiency of the latter organ must necessarily enter largely into the question.

Later the time of the appearance of the dye in the bile was estimated (83).

All the methods so far proposed depend on recovery of dye after injection and excretion by either the liver or kidney, and all have their drawbacks. An important advance was, therefore, made by Rosenthal (84), who approached the problem from a new angle - namely, estimating the rate at which the dye leaves the blood-stream. This worker noted that in dogs with a damaged liver a higher concentration and a longer retention of the dye occurred than in ones with the organ in a normal condition. He further makes the important statement that 12% of the liver must be excised before a positive result was obtained by this method. Obviously, in the case of an organ with such a large reserve as the liver, a test which would reveal results when only 12% was thrown out of action, would be a procedure of great value. It was found that in doses of 5 mg. per kilo of body weight, the dye was normally almost or quite absent from the blood in one hour after injection. He considered there was evidence that curves obtained in hepatic disease showed a parallelism with the degree of derangement. Curves worked out by Rosenthal for various liver conditions are reproduced in Figs. 10 & 11 (85). There are, however, several very potent disadvantages of this method. The

injection of the dye has been known to result in death, and lesser dangers are thrombosis, induration at the site of the injection, rigors and chills, etc. It is impossible to regard with equanimity a test that may at any time result in dangerous complications.

It is also deficient in other respects. For instance, it is of no value in certain types of jaundice, (especially obstructive) as well as in certain other liver conditions (21).

Other dyes have been tried at various times, e.g. Indigo-Carmine, which was first used systematically in liver investigations by Hatiegamu (86) and Einhorn and Laporte (87); methylene blue (88); congo red (89) etc. More recently such new dyes as rose bengal (90) and azorubin S. (91) have been introduced. Rosenthal and White (92) have also sponsored a new dye which they describe as "ideal for the purpose of testing liver function." Normally this substance is removed rapidly by the liver. After excision of this organ they found that "it is retained in the blood serum almost in toto during the early period following injection." The dosage is smaller than that of phenotetrachlorophthalien, viz: 2 mgrm. per kilo of body weight. Five and thirty minute blood specimens are examined colorimetrically for the

percentage of dye. The claim is advanced that the percentage of dye retained after half-an-hour represents directly the degree of hepatic inefficiency.

Rose bengal, which has been introduced as a liver efficiency test by Delprat and his co-workers, has one advantage in that the blood samples are taken in 3, 4 and 8 minutes and the calculations completed in one hour. Unfortunately these calculations are of an involved nature and the authors themselves only claim that "a rough estimate can be formed of the extent of liver disease."

Tada and Nakashima (91), who suggested azorubin S. as "the best dye for clinical use", tried no fewer than 62 different substances before they came to this conclusion. In their paper they point out the disadvantages of methylene blue, phenoltetrachlorophthalein, etc. They define the ideal dye as "one that does not appear in normal bile or one that is eliminated entirely by the liver." Azorubin S. is "extremely harmless" and very stable. It is easy to determine its first appearance in the bile and its concentration here is very high. In the dog 95% is excreted in the bile and the rest in the urine. If "the eliminating function of the liver" is disturbed then the "duration of the dye in the urine is greatly prolonged."

DISCUSSION OF DYE TESTS.

DISCUSSION OF DYE TESTS.

DISCUSSION OF DYE TESTS.

Theoretically the principle of dye tests in general seems to be promising, with this reservation, that the liver is called upon to deal with a substance entirely foreign to its experience. It is, therefore, possible that the organ may not act towards it uniformly or proportionately under the varying conditions of health and disease. At this stage there is little that can be said about dye tests beyond the fact that they are entirely in the experimental phase. Much experience will be required before their value can be assessed. Certainly the earlier dyes have proved unsatisfactory in various ways. Furthermore, any dye that is to be estimated in the urine introduces the question of renal efficiency, which must weaken its claims very much. There is also the difficulty that in the event of the liver cells being unable to pass on the dye to the bile capillaries we have by these tests no means of differentiating this state of affairs from direct obstruction lower down. Bromsulphalein seems to have the most possibilities of usefulness on present indications.

None of the dye tests have been much worked at in this country. In conclusion, we may refer to an interesting application of certain of these dyes, which

being opaque to X-rays have been utilised as a means of visualising the gall-bladder, a procedure introduced by Graham (93).

(VI) TESTS BASED ON PALMISTOGEN SULFONATE

(VI) TESTS BASED ON HAEMOPOIETIC FUNCTIONS.

(VI). TESTS BASED ON HAEMOPOIETIC FUNCTIONS.

Before concluding the list of tests for hepatic function, it remains to refer to one or two which are classified under the above heading. The first is the -

"FIBRINOGEN TEST"

It has been demonstrated in animals that fibrinogen disappears from the blood after extirpation of the liver and that it is diminished in Phosphorus and in Chloroform poisoning. Methods of estimating its quantity in the blood have been devised, and it is reported to be normally fairly constant in range in the human blood (385 to 618 mgrm. per 100 c.c. - (94) and 250 - 400 mgrm. per 100 c.c. with average about 330 mgrm. (95) being suggested figures). It is claimed by McLester and his collaborators (95) that a high figure is obtained in diseases "stimulating" the liver and a subnormal result in ones which "depress or destroy a large part of the liver." They include a number of charts, but think that the method is not yet sufficiently refined for diagnostic application.

COAGULATION TIME OF BLOOD.

It is said that this is increased in hepatic diseases, but the method has not yet been sufficiently tried for an estimate of its worth to be made.

SECTION V.

SUMMARY AND CONCLUSIONS.

SUMMARY AND CONCLUSIONS.

In the foregoing an attempt has been made to evaluate the more important of the tests suggested for estimating the several hepatic functions. A considerable amount of literature has been consulted in the endeavour to ascertain the views of various authorities in the different countries (either directly or through the medium of resumé's in English in the case of foreign literature). On reading these it appeared to the writer that, in view of the legion of tests suggested from time to time, a useful purpose could be served by bringing together the chief arguments, in order to decide, if possible, whether any definite advantages are likely to accrue from continued research with many of them. It seemed to him that there was a tendency rather to keep adding to a list already encumbered, and so creating more and more confusion. From the outset it is obvious that the liver efficiency tests as a group must be regarded as still very much in the experimental stage, and evaluated accordingly. In the introduction we have outlined the nature of the chief difficulties that beset the investigation of hepatic functional capacity. One of the disadvantages referred

to was the need for a plurality of tests, as we are dealing with an organ possessed of a wide range of functional activities whose inter-relationships are by no means worked out. The outline of the tests given in Section IV shows that we are at present quite unable to rely on any one as a general test of the liver functions. Indeed, until the minute physiology of the organ is much better understood, it will scarcely be possible to dispense confidently with all but one or two.

But the problem of finding one or two tests which will suffice for most practical clinical purposes is not, by any means, a hopeless one, though admittedly fraught with great difficulty. It is only to be expected that in the case of any organ with such a large reserve, with such a potential power of hypertrophy and hyperplasia, and with so much tissue capable of being attacked by disease in varying degrees of intensity, we will obtain anomalous results with function tests. Only very wide experience will enable us to evaluate their results in proper perspective.

Taking into consideration the main aim of these tests, that of clinical utility in helping diagnosis of early or difficult cases, in aiding prognosis or treatment, we may summarise our views of the method

above outlined as follows:-

Metabolic Tests.

The Laevulose Test promises to be a valuable test. There is as yet no proved advantage of the galactose technique over the Laevulose one. The Protein and Fat Tests are, in their present form, valueless.

Pigmentary Tests.

The presence of pigments in the urine or their absence from the faeces are gross indications of hepatic or biliary disorder.

Van den Bergh's test has proved disappointing. The quantitative reaction is valuable for estimating the bilirubin content of the blood, especially in early cases, "latent jaundice" etc.

Urobilinuria usually point to hepatic disorder, though also found in haemolytic conditions and probably in certain cases of intestinal stasis.

Secretory Tests.

Bile Salts in urine point to obstruction to biliary outflow. Their absence in later cases of this condition suggests gross liver damage.

Blood-Cholesterol estimations have given such discordant results that ^{they} are of no present help.

Antitoxic Tests.

None of the present methods is sufficiently refined to be of value.

Excretory Tests.

The dye tests are promising but it is too early to assess their usefulness. They will always have the weakness of not yielding any information about the condition of the liver in obstruction of the biliary passages.

Haemopoietic Tests.

Are of no clinical value at the moment.

We, therefore, believe that a drastic cut in the number of hepatic efficiency tests, of which the number considered is but the more feasible portion, is indicated,

Of those above enumerated it seems to the writer that the ones chiefly deserving of attention are the laevulose Test, the Dye Method and the Van den Bergh's quantitative method.

The Dye tests have obvious limitations. Van den Bergh's quantitative test is already a valuable procedure in certain cases.

The laevulose test certainly seems to warrant attention. We believe that it promises to be a more delicate test than at first might seem apparent, and would,

therefore, prove of value in diagnosis, prognosis and treatment.

But in connection with all these methods too little is known of the response in large numbers of normal persons as well as of pathological cases under the conditions of the test. Without such material it will not be possible to make any sound comparisons in health and in disease, to weigh up the effect of possible fallacies, and finally to estimate the position of such a test in its relation to the problem of general hepatic functioning efficiency.

CONCLUSIONS.

(1) A number of suggested hepatic efficiency tests have been considered and after due consideration of the results obtained, it is suggested that many of them are at present of little or no value as an aid to the clinician, owing to physiological or technical faults, or to their grossness, etc.

(2) The Laevulose Test, the Dye Tests and Van den Bergh's Quantitative Test are considered to be the ones most promising in respect of clinical utility.

(3) The Laevulose Test promises to be an especially valuable procedure.

(4) That the tests are mostly in the experimental state and it will be necessary to apply them to a large number of cases, in health and in disease, to enable an estimate of their true value to be arrived at.

SECTION VI.

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SECTION VII.

DIAGRAMS AND CHARTS.

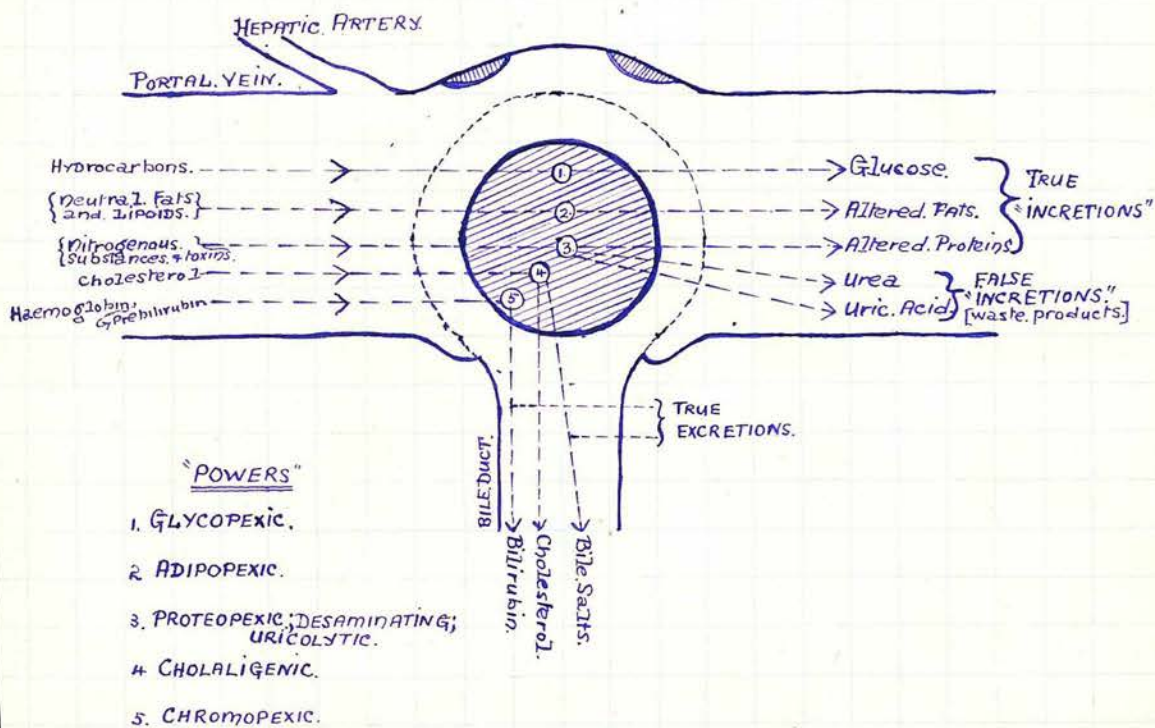


Fig. 1. Diagram of Liver Functions.

(After Gruner)

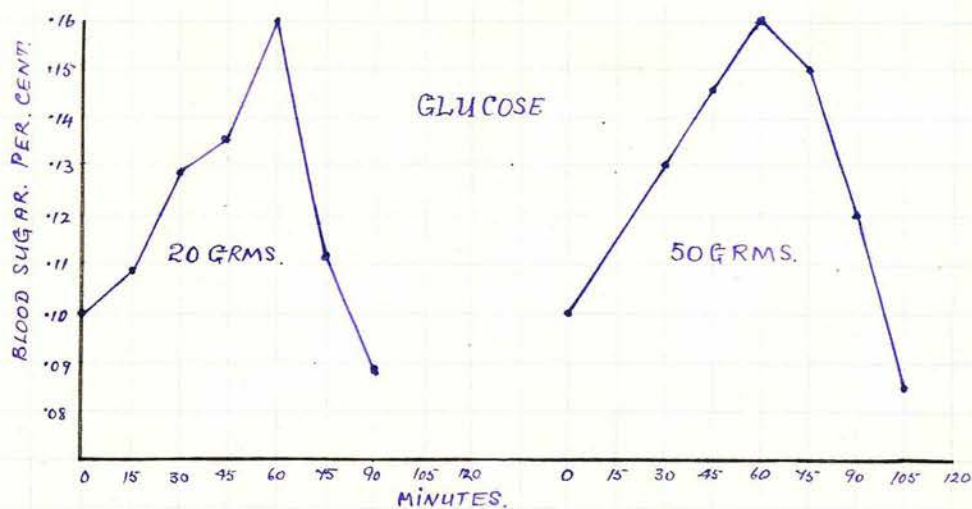
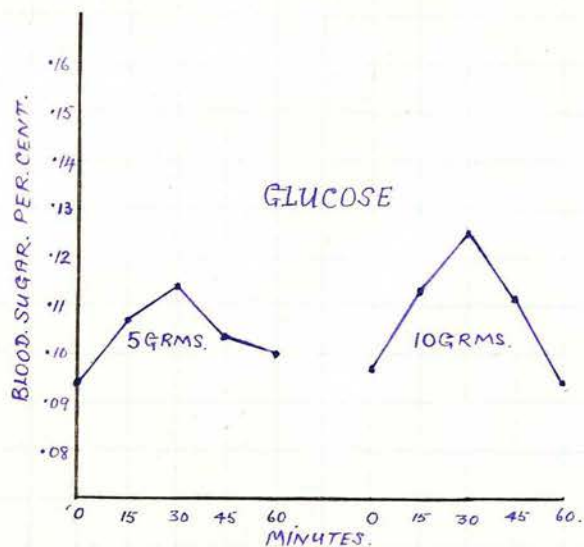


Fig. 2. Blood Sugar Curves following ingestion of Glucose. (After Maclean)

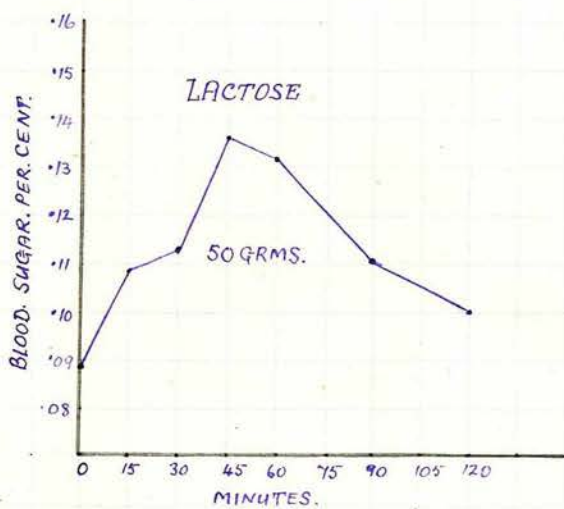


Fig. 3. Blood Sugar Curve after ingestion of
Lactose. (After Maclean).

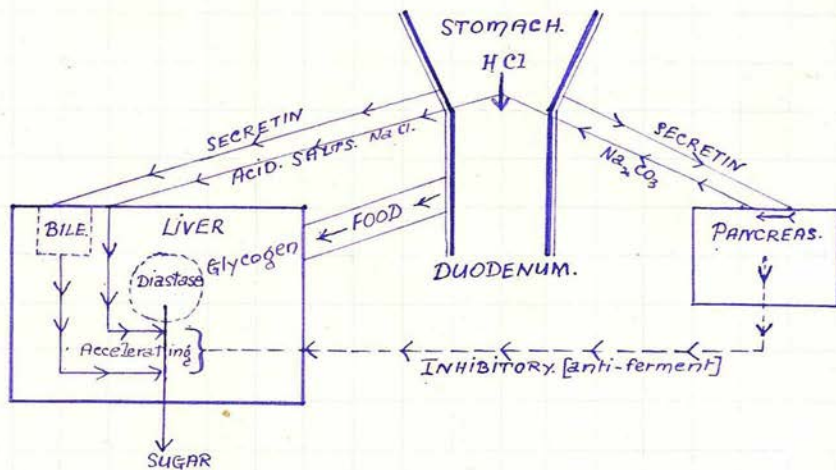


Fig. 4. Diagram illustrating factors controlling blood-sugar content, according to Cammidge.

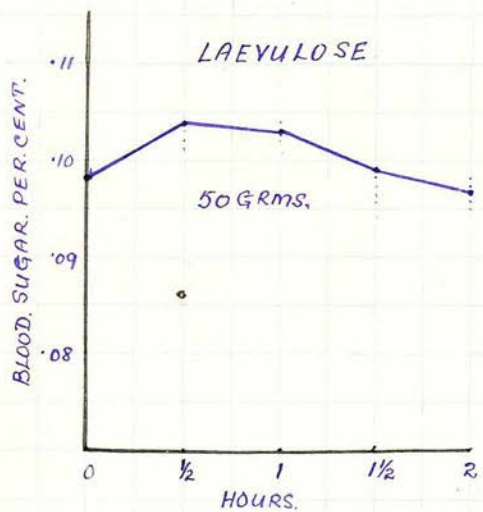


Fig. 5. Blood-Sugar curve after ingestion of
laevulose in normal subject.

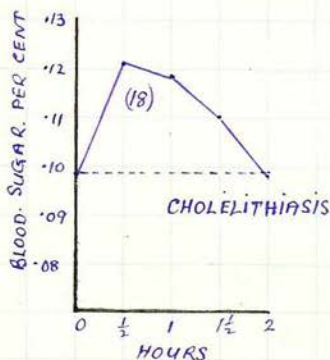
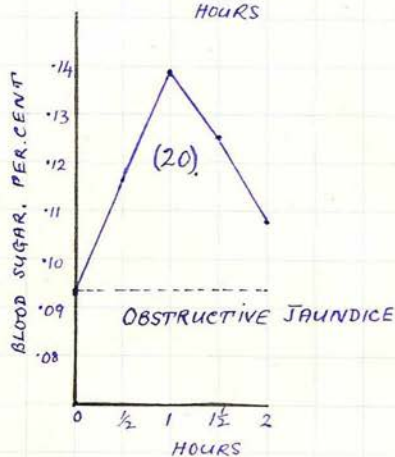
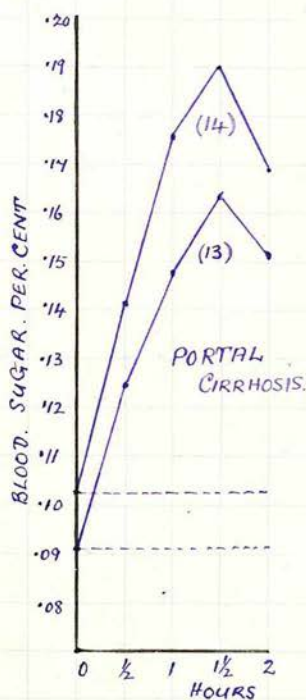
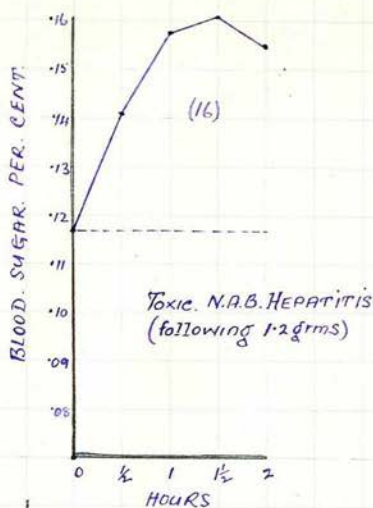
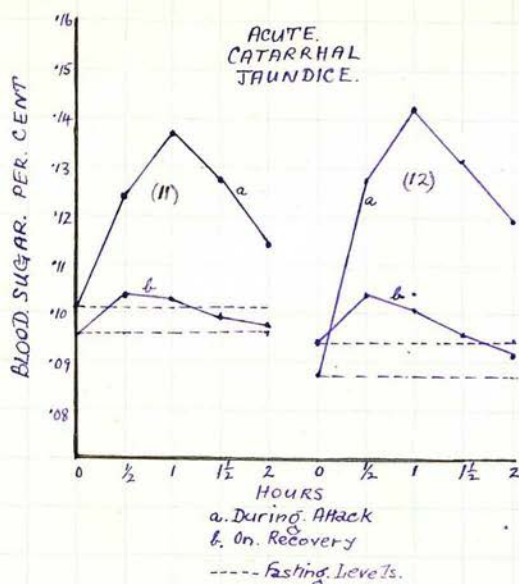


Fig. 6. Blood-Sugar curves after ingestion of Laevulose in various hepatic conditions.

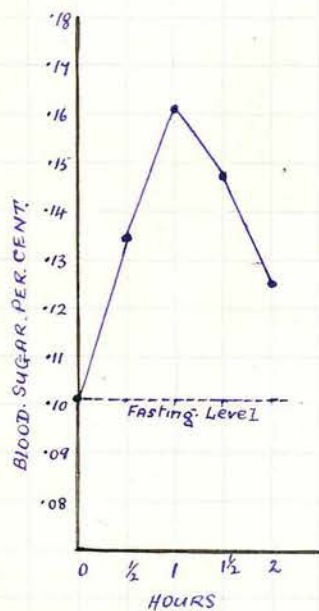


Fig. 7. Blood Sugar Curve after Laevulose
in Pernicious Anaemia.

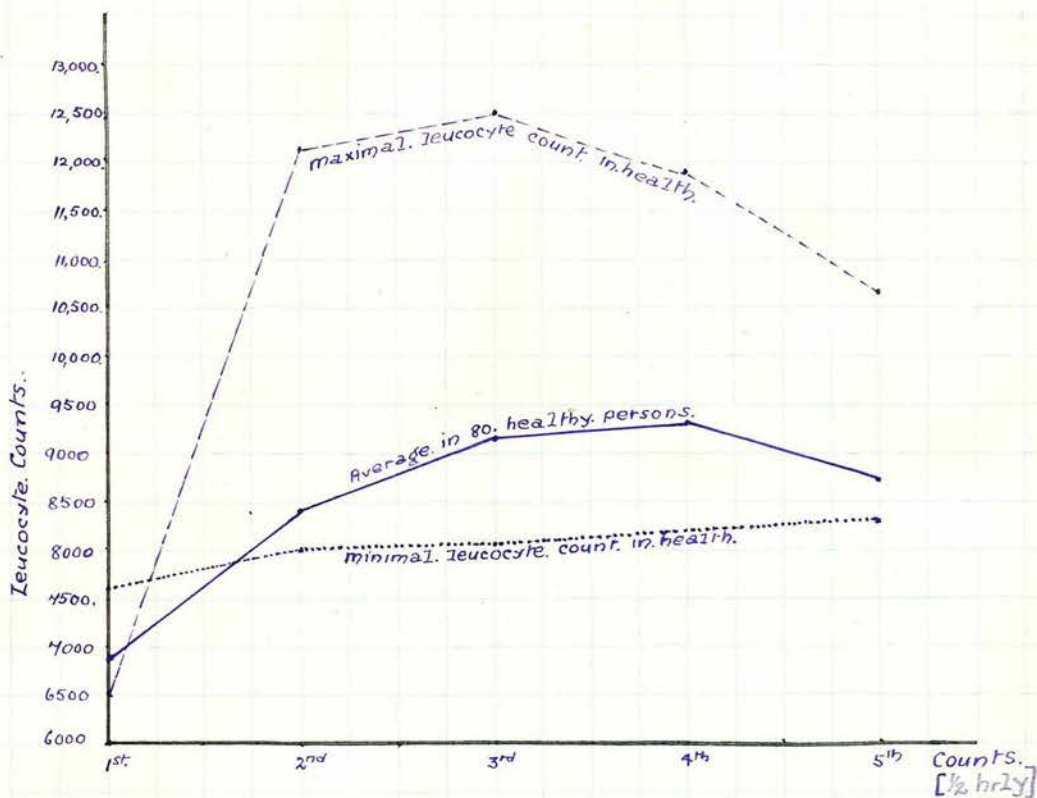


Fig. 8. Leucocytic blood counts after 200 c.c. of milk in normal subjects. (After Feinblatt).

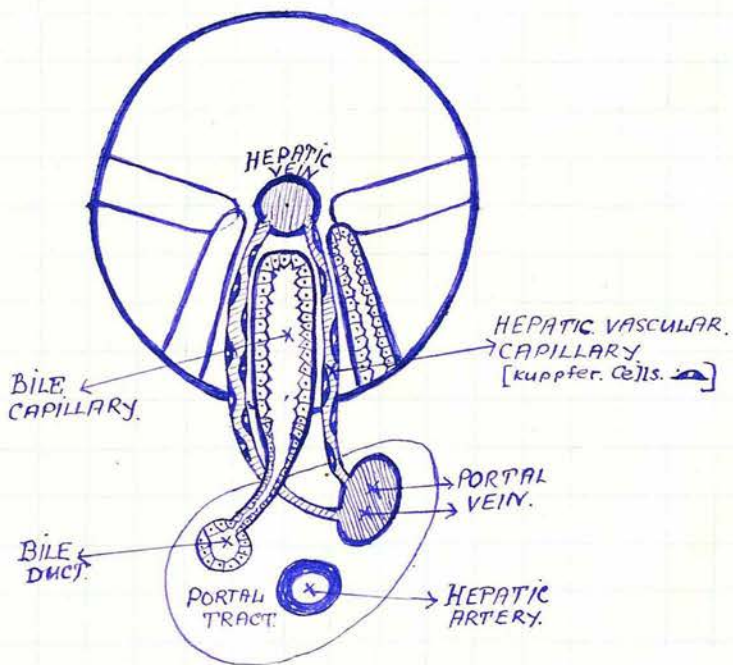
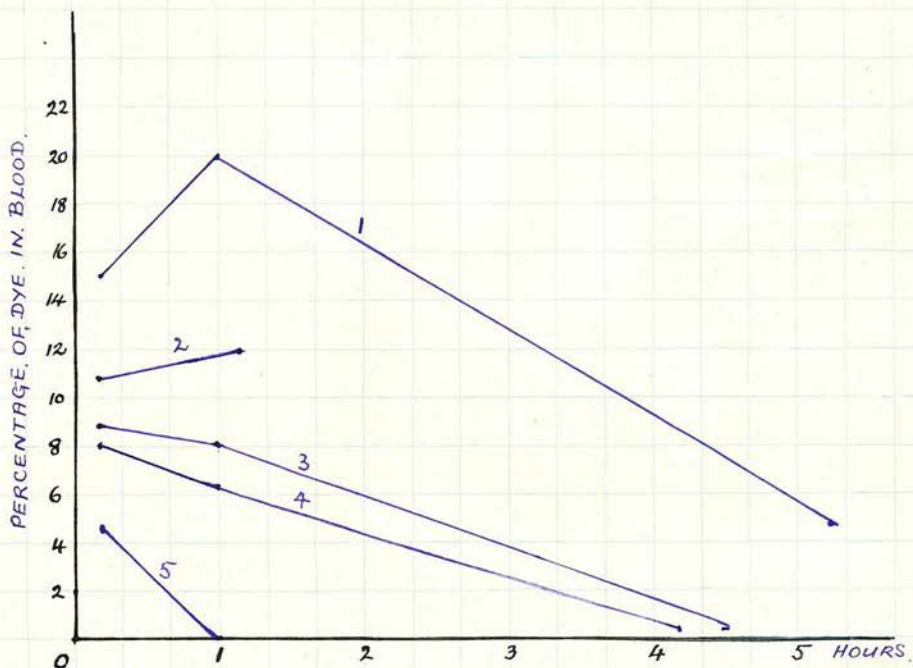


Fig. 9.

Diagram of Liver Lobule.

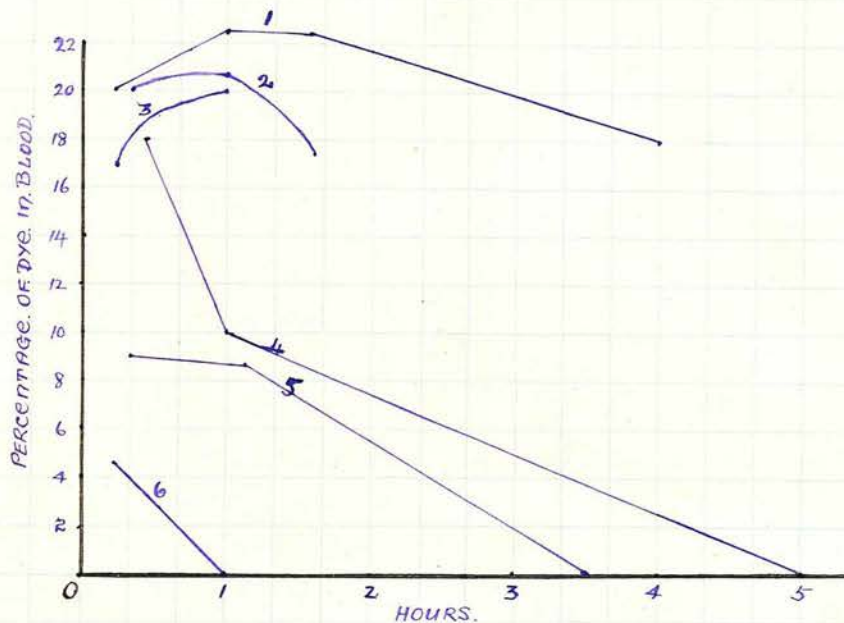
(After McNee).



1. Atrophic Cirrhosis
2. Cirrhosis in child [Hanot Type]
3. Hypertrophic Cirrhosis
4. Ditto - three weeks later
5. Normal Curve.

Fig. 10. Phenoltetrachlorphthalien Test for Liver Function - Curves in Cirrhosis of Liver.

(After Rosenthal)



1. ACUTE HEPATITIS [? ETIOLOGY]
2. " " " [following. Arsphenamine]
3. CHOLANGITIS - probably. Acute. Hepatitis.
4. SOLITARY LIVER. ABSCESS.
5. CHRONIC. PASSIVE. CONGESTION. -degenerative changes. in. Liver.
6. NORMAL.

Fig. 11. Phenoltetrachlorphthalien Test for Liver Function - Curves in various Liver Diseases. (After Rosenthal).